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Resistance to Therapies for Infection by *Plasmodium vivax*

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INTRODUCTION

The scope and depth of this review reflect the complexity of therapy for malaria in general and of *Plasmodium vivax* in particular. Even for malariologists steeped in an understanding of the treatment of acute, uncomplicated malaria caused by *Plasmodium falciparum*, *P. vivax* challenges. The tendency to fold the two species together into a single conceptual framework, and they indeed share some common ground, leads to errors in both strategic reasoning and practice with therapies. These two species are biologically and clinically distinct in fundamentally important ways. This review attempts to draw a distinction with *P. vivax* in the broad context of chemotherapies and resistance to them. This task carries the necessity of detail permitting a grasp of therapeutic strategies, the problem of resistance to those therapies, and an appreciation of the singular nature of these among human malarias. The review gathers and links seemingly disparate facets of biology, epidemiology, and clinical science of the infection along with key aspects of the pharmacology of the drugs arrayed against it. The aim is an improved likelihood of the effective application of science against these parasites, which are responsible for so much human suffering.

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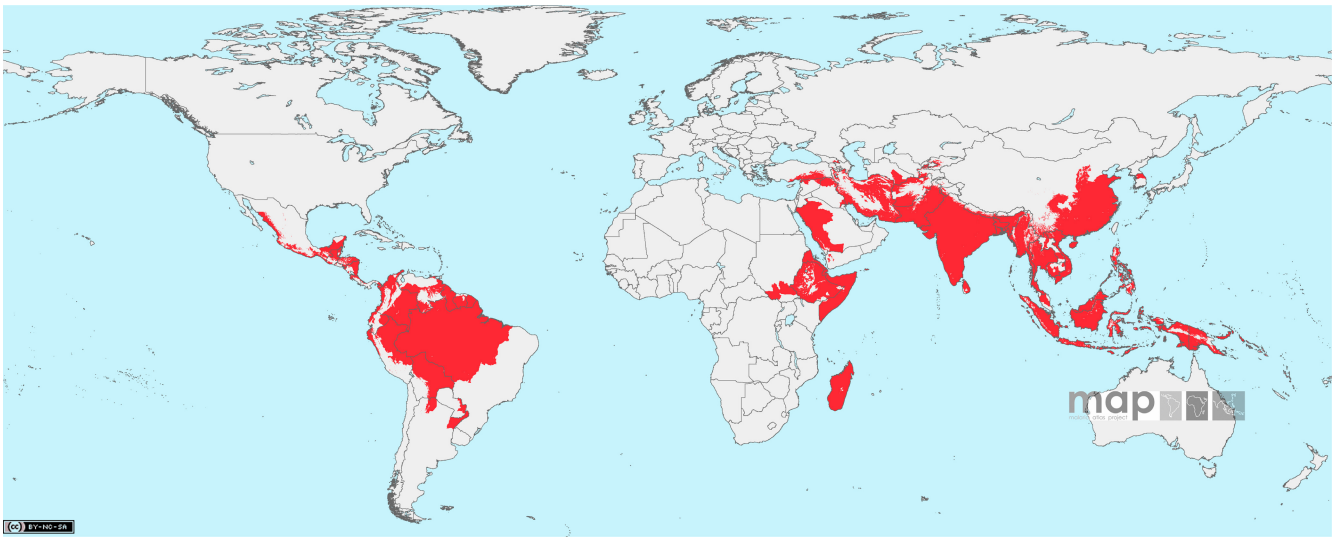

FIG. 1. Biological limits of the global distribution of *P. vivax* based on temperature, humidity, aridity, and contemporary reports of malaria risk. (Courtesy of Carlos Guerra, Malaria Atlas Project, Oxford University, United Kingdom.)

VIVAX MALARIA

A Neglected Infection

The perception of vivax malaria as infecting relatively few people, causing a benign course of disease, and being treatable with widely available therapies largely explains the almost complete neglect of this important infection (20). The two frontline drugs, chloroquine and primaquine, are each more than 50 years in service, their mechanisms of activity against the parasite are not understood, and standardized means of ascertaining parasite resistance to either drug do not exist despite almost 20 years of mounting evidence of resistance (18). In the case of primaquine against dormant liver stages, for which no sure therapeutic alternatives exist, no clear evidence demonstrates that the drug remains effective as prescribed (19). Moreover, toxicity and dosing issues with primaquine severely limit its utility in the endemic rural tropics (21).

Evidence is growing that *Plasmodium vivax* may infect many more people than has been appreciated (102, 172). Evidence also points to this supposedly benign parasite causing a spectrum of severe, life-threatening syndromes that are strikingly similar to those caused by *Plasmodium falciparum* (20, 22, 88, 119, 172, 224). The inadequacy of the therapeutic tools to cope with this threat encompasses the inability to gauge effectiveness and efficacy (20). The majority of the many millions of people infected by *P. vivax* this year will be treated with chloroquine. The risk of treatment failure with this drug in any more than a few areas of endemicity remains unknown. One study in Indonesian New Guinea put that risk at nearly100% (210), and many other studies in that region reported that it exceeded 50% (12, 14, 15, 180, 211). Surveys in Thailand and India, however, showed uniform sensitivity to chloroquine (125, 136, 154, 229), except for a recent report from Gujarat in western India, showing a 9% failure rate (209). The experience with *P. falciparum* drug resistance allows a reasoned forecast of the further deterioration of efficacy where the problem now occurs and its encroachment toward the Indian subcontinent, the nexus of the global burden of vivax malaria. The establishment of chloroquine-resistant vivax malaria in that region seems likely to occur well before any effort to field a vaccine against this parasite, severely stunted in development relative to falciparum malaria, yields such a tool.

Global Burden

Plasmodium vivax stands alone in its geographic versatility among the other four species of plasmodia infecting humans. *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi* do not now occur beyond the tropics, and *P. falciparum* does so only sporadically. Endemic *P. vivax* occurs throughout the tropical latitudes, excluding the South Pacific east of Vanuatu, where anopheline mosquitoes do not appear, and most of western and central Africa, where the absence of Duffy factor on human red blood cells apparently prohibits this parasite from an endemic distribution (63). Vivax malaria extends well beyond the tropics, through most of eastern China up to and including the Korean Peninsula. It occurs all along the tropical and temperate southern fringe of Asia, reaching to the Mediterranean Sea. Figure 1 illustrates this distribution. The sporogonic development of *P. vivax* in anopheline mosquitoes cannot occur at temperatures below 15°C, and latitudes having summer isotherms near this limit represent the most likely limits of its biologically possible range (51). According to Guerra and colleagues, 2.6 billion people live at risk of infection by *P. vivax* (93). The estimated number of annual clinical cases ranges from 70 million (147) to 390 million (102, 172). The overwhelming majority (probably >80%) of these cases, whatever the actual total, occur in densely populated South and Southeast Asia, from where mounting evidences of both drug resistance and severe disease now emanate. Although the burden of vivax malaria in the Americas is probably relatively light, it accounts for more than 70% of malaria cases in that region (31, 147).

A Malignant Infection

Early malariologists distinguished *P. vivax* from *P. falciparum* and *P. malariae* on the basis of clinical course, with *P. vivax* and *P. falciparum* causing paroxysmal fevers at intervals of about 48 h and *P. malariae* having intervals of 72 h. The first two thus became known as tertian malaria, and the latter became known as quartan malaria (237). The relatively pernicious course of *P. falciparum* compared to that of *P. vivax* separated these two species, thus called malignant and benign tertian malarias, respectively. The recognition of *P. ovale* as a species distinct from *P. vivax* came decades later, and an appreciation of *P. knowlesi* as routinely infecting humans came only in 2007 (61). Even though malariologists have long preferred terminology applying the specific epithet of the Linnaean binomial species to the common name for the infection, i.e., falciparum malaria and vivax malaria, etc. (237), the use of the older clinical terminology persists.

The term benign applied to vivax malaria had always been in the context of being relative to falciparum malaria. Few who have endured vivax malaria would characterize the experience as being benign. Fatal cases of vivax malaria, although rarely documented, have certainly been known and should dismiss the notion of "benign" as having been applied in any absolute sense. Comparing relatively early stages of disease, clinical symptoms of vivax malaria may be markedly more uncomfortable than those of falciparum malaria (235) and may provoke higher levels of inflammatory cytokines per parasitized cell (103). Nonetheless, authoritative references in medicine and science available in 2008 asserted that *P. vivax* only rarely causes death and does so by infarct or rupture of the spleen. No text links vivax malaria with severe cerebral, pulmonary, renal, or hepatic syndromes, but recent evidence challenges that perspective.

The biology of *P. vivax* in its vertebrate host has been viewed through the lens of almost always being relatively benign to humans. Biological distinctions between *P. vivax* and *P. falciparum* became, de facto, contrasts in the consequences of distinct parasitism strategies employed by the plasmodia in humans. The casting of contrasted consequences of falciparum and vivax malarias provided a useful analytical lever. The presence of a trait in falciparum malaria and its absence from *P. vivax* made it suspect as a determinant of disease severity. Likewise, shared characteristics were often disqualified as determinants of severe disease. The utility of this lever, and the dogma derived from it, would be doubtful if evidence emerged to demonstrate a spectrum of severe disease syndromes among patients with vivax malaria resembling that among patients with falciparum malaria.

Observations from the field within the past 5 years indeed challenge the notion of vivax malaria as being a rare and fastidious killer. Reports of vivax malaria marked by delirium, seizures, renal failure, shock, hepatic dysfunction, severe anemia, lung injury, pulmonary edema, and acute respiratory distress have come from South and Southeast Asia, the Middle East, and South America (23, 24, 36, 108, 120, 122, 128, 131, 139, 140, 146, 149, 150, 160, 170, 181, 182, 193, 206, 208, 215, 216, 222, 225, 228, 240), including one case of *Plasmodium ovale* (182). Some of those reports (24, 36, 128, 139, 170, 193, 206, 240) applied PCR diagnostics to rule out cryptic or misdiagnosed falciparum malaria infection of these patients, and some effectively ruled out other infectious agents. Another study reported bacteremia with *Salmonella* as being the primary cause of fever, shock, jaundice, and renal failure in a patient with concurrent vivax malaria (168). Splenic rupture or infarct with vivax malaria indeed occurs (89, 116, 159, 161) but is either very rare or poorly represented in the medical literature.

The only recent application of PCR technology to discriminate causes of malaria in endemic settings represents the lynchpin unhinging the conviction that vivax malaria very rarely causes life-threatening disease. Clinicians treating patients for delirium, coma, seizures, respiratory distress, renal failure, or jaundice caused by malaria would have great difficulty drawing upon any training or text to support a diagnosis of vivax malaria. Reliable microscopic diagnostics, even if available, would rarely discourage the diagnosis of falciparum malaria. The ability to rule out falciparum malaria by using PCR technology eliminates the default assumption of *P. falciparum* parasites being present but sequestered within the deep organs of such patients. An urgent need exists for gross and histological postmortem examinations of patients dying with a diagnosis of vivax malaria.

Vivax malaria seems to cause severe disease syndromes that closely resemble those historically attributed to falciparum malaria. The rare clinicians having both the luxury of diagnostic certainty and experience with the malarias may assert the relatively benign nature of vivax malaria with respect to the likelihood of fatal outcomes. This represents what likely amounts to a unique perspective, i.e., that of vivax malaria among otherwise healthy people experiencing a rare or singular event. In the broader view of endemic vivax malaria occurring almost universally among people lacking access to even modest clinical investigation capacities, patients may present constellations of genetic, immunological, nutritional, and coinfection factors leading to a lethal course of infection. The challenge for the science of clinical malaria is to understand the circumstances under which vivax malaria turns deadly, and this may well include the problem of resistance to therapies.

Natural History

The tenacity of vivax malaria in temperate areas like the Korean Peninsula, with long frigid seasons incapable of supporting mosquitoes, lies in its ability to place dormant stages in the liver. When an infected anopheline mosquito bites a human, typically dozens of sporozoites traffic to the liver and probably most eventually take up residence in hepatocytes. Unlike all of the other human plasmodia except *P. ovale*, a largely unknown and probably variable fraction of those parasites develop into a quiescent hypnozoite rather than an actively dividing tissue schizont. The active primary tissue schizont matures in about 7 days, and the release of merozoites into the bloodstream prompts the onset of acute malaria, usually within a few days. The hypnozoite, taking an unknown later cue, blooms into an actively dividing tissue schizont with the same consequences of bloodstream infection and clinical malaria, called a relapse (124). Figure 2 illustrates the life cycle of *P. vivax*.

The timing and risk of relapse vary with local climate. Strains

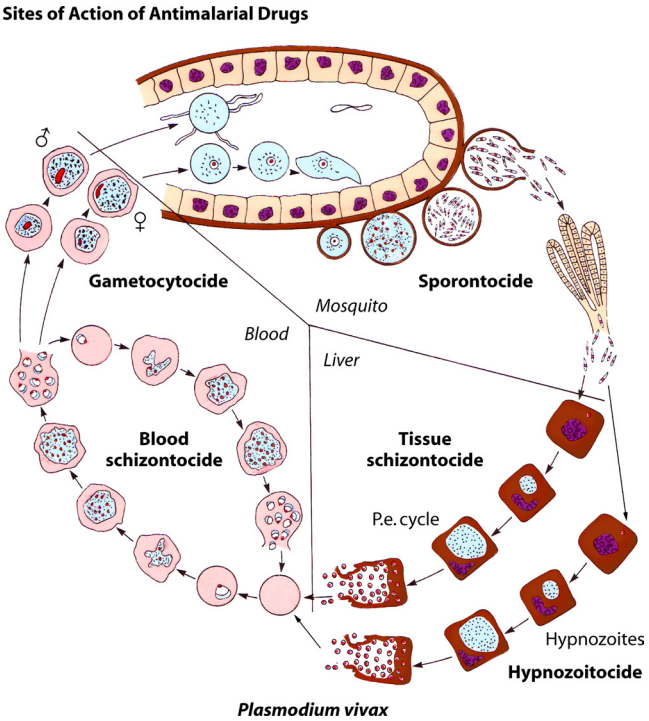

FIG. 2. Compartments of antimalarial activity shown within the life cycle of *P. vivax*. At left, a mosquito ingests infectious gametocytes, which become infectious sporozoites. This sporogonic arm of the cycle may be attacked with drugs called sporontocides. Sporozoites invade the liver and become either dividing tissue schizonts or a dormant hypnozoite that later becomes another dividing tissue schizont. This arm of the cycle may be attacked with tissue schizontocides and hypnozoitocides. The tissue schizonts release merozoites into the blood, and these infect red blood cells and commence growth into trophozoites, dividing and producing mature schizonts full of merozoites that are capable of invading still more red blood cells. This arm of the cycle, which is responsible for clinical malaria, can be attacked with blood schizontocides. Some merozoites from the liver that invade red blood cells do not become asexual blood schizonts but instead differentiate into male and female sexual forms called gametocytes. The infectiousness of gametocytes to mosquitoes may be attacked with drugs called gametocytocides. P.e., pre-erythrocytic. (Courtesy of Wallace Peters and Andrea Darlow.)

from areas having seasons that are inhospitable to anopheline mosquitoes tend to relapse infrequently, at long intervals, and only once. The timing of relapse seems to correspond to the seasonal local abundance of anophelines. In other words, the dormant parasite launches itself into the bloodstream when the odds favor encountering a feeding anopheline where sexual recombination can occur. That event marks the anopheline mosquito as the definitive host of plasmodia. Humans, as intermediate hosts, serve merely as a vessel for mitotic amplification that improves the odds of infecting another mosquito. At one extreme of climate, *P. vivax* in Northern Asia (perhaps a subspecies and called *P. vivax hibernans*) lacks a primary bloodstream parasitemia. It emerges into the bloodstream only after 1 year, almost certainly as a result of the selection pressure exerted by the extraordinarily brief opportunity for anopheline mosquitoes to exist in that climate. A well-characterized North Korean strain of *P. vivax* represents this type (198). The period may be too brief for achieving sexual recombination, sporogonic development, and infection of yet another human in whom the parasite may survive through a long winter. At the other extreme of climate is a tropical Asian strain like *P. vivax* Chesson from perpetually warm and wet New Guinea. This parasite relapses quickly (about 18 days from the onset of primary parasitemia) and three or more times at brief intervals of a few weeks (47). There is no temporal window of anopheline abundance through which parasites in New Guinea must pass. Thus, in cooler-temperature latitudes, promiscuously relapsing parasites were likely eliminated by the absence of a suitable invertebrate host, and strains programmed for discrete, well-timed relapse prevailed. There are no known extrinsic cues for plasmodia to relapse, and tropical strains evaluated in temperate climates follow their apparently intrinsically programmed relapse behaviors.

If one considers disease to be the defining event of infection, the hypnozoite may be considered a reservoir of new infection rather than a dormant old infection. One study in Papua New Guinea (115) presented evidence suggesting that the hypnozoite represented the overwhelmingly dominant source of new parasitemias relative to new infections (21). Chemotherapeutic attack on the hypnozoite thus resembles conventional malaria control measures aimed at reducing new infections, i.e., those minimizing human contact with anopheline mosquitoes by applying indoor residual insecticide spraying and bed nets, etc. Malaria control and certainly eradication strategies failing to recognize the hypnozoite as a source of new parasitemias may meet with little success. This view emphasizes the central importance of treatment and drug resistance in vivax malaria with regard to strategies for controlling its transmission.

Relapse profoundly impacts strategies for the prevention, treatment, and control of vivax malaria. It imposes the necessity of two classes of drugs: (i) treatment of acute malaria and (ii) elimination of the liver stages to avoid subsequent relapse. Relapse also imposes nagging ambiguities in understanding therapeutic efficacy and drug resistance, a problem severely compounded by the failure to adapt this parasite to continuous culture. In vitro culture systems would permit direct and objective assessments of drug activity against well-characterized strains of the parasite, but in the absence of these tools, such assessments must be done using more difficult, costly, and sometime ambiguous ex vivo and in vivo systems.

THERAPY OF VIVAX MALARIA

Malaria Chemotherapeutics

The unique terminology of malaria chemotherapeutics reflects complexities in describing classes of drugs targeting specific stages of the plasmodia. In general, a single therapeutic regimen of an antimalarial drug targets only a single segment of the life cycle of the parasite. For example, mefloquine is administered against asexual blood stages, and other drugs must be administered against sexual blood stages and asexual liver stages. Some drugs, however, exhibit a broader spectrum of activity and may serve a dual purpose; e.g., a therapeutic regimen of primaquine aimed at asexual liver stages will also serve to neutralize mature extant sexual gametocytes and prevent transmission to mosquitoes. However, a regimen of primaquine aimed only at the sexual blood stages (a single 45-mg

TABLE 1. Classes and representative antimalarial drugs[a]

Type of drug	Target	Clinical application	Prophylaxis	Licensed drug(s)	Experimental drug(s)
Blood schizontocide	Trophozoite in blood	Treatment of acute malaria	Suppressive	Chloroquine, quinine, mefloquine, doxycycline, AV-PG, DH-PP	Tafenoquine, ACTs
Primary tissue schizontocide	Active schizont in liver	None	Causal	Primaquine	Tafenoquine
Hypnozoitocide	Dormant hypnozoite in liver	Prevention of relapse	None	Primaquine	Tafenoquine, elubaquine
Gametocytocide	Gametocyte in blood	Prevention of transmission	None	Primaquine	Tafenoquine, artesunate, artemether
Sporontocide	Forms in mosquito including sporozoite	Prevention of transmission	Causal prophylaxis	Primaquine	Tafenoquine

[a] AV-PG, atovaquone-proguanil (Malarone); DH-PP, dihydroartemisinin-piperaquine (Artekin); ACTs, artemisinin combined therapies (many in evaluation).

dose) is inadequate to effect a cure of liver stages. The stage specificity of drugs may also vary with species of plasmodia; e.g., therapeutic doses of primaquine (aimed at asexual liver stages) also kill the asexual blood stages of *P. vivax* (175) but not those of *P. falciparum* (11). Likewise, chloroquine (aimed at asexual blood stages) also neutralizes the infectivity of mature *P. vivax* gametocytes to mosquitoes (111) but not those of *P. falciparum* (35). These examples illustrate the necessity of considering therapeutic intent, dose, species, and stage targeted in classifying a specific drug among classes of antimalarials.

Table 1 lists the classes of antimalarials and their essential characteristics, and Table 2 lists and defines the terminology often used in the context of malaria chemotherapeutics. When malariologists refer to a radical cure of vivax or ovale malaria, they infer the application of two or more drugs aimed at the elimination of blood and tissue stages of plasmodia. A radical cure of falciparum or malariae malaria can be achieved with a single drug. Suppressive and causal prophylactic antimalarials refer to the prevention of malaria using blood schizontocides or tissue schizontocides, respectively. A suppressive prophylactic does not prevent the root cause of clinical malaria, infection of the liver, whereas a causal prophylactic does. Travelers taking suppressive prophylaxis are often directed to take a standard course of primaquine therapy aimed at liver stages in order to prevent the subsequent relapse of vivax or ovale malaria. This treatment is often called prophylaxis, but the term presumptive antirelapse therapy is preferred (105).

Another set of important terms often used by malariologists bears directly upon therapy. The biology of the term relapse has been explained as an acute blood-stage infection originating from a hypnozoite. This term contrasts with parasitemias originating from reinfection or recrudescence. These terms represent the three possible sources of patent blood-stage infection following therapy of a primary parasitemia. Reinfection obviously represents an event arising from a separate exposure to mosquitoes carrying sporozoites. Recrudescence describes the patency of blood stages driven to subpatency and subsequently reappearing. Another term is often applied to describe patency following treatment: recurrence. This term is deliberately ambiguous with respect to the origin of the parasitemia, and it is applied when the source of a new parasitemia is uncertain.

Chloroquine Therapy

Chloroquine has been the therapy of choice for the treatment of acute vivax malaria since 1946 (151). Developed as resochin in the 1930s, its German developers abandoned the drug in favor of a seemingly less toxic methylated analog called sontochin. The U.S. Army obtained experimental sontochin tablets in Algeria during late 1943 and almost immediately

TABLE 2. Malaria chemotherapeutic terminology

Term	Definition
Blood schizontocides	Drugs aimed at asexual blood-stage parasites to effect a cure of clinical malaria
Primary tissue schizontocide	Drug aimed at primary asexual liver-stage parasites to prevent primary blood infection
Hypnozoitocide	Drugs aimed at latent asexual liver-stage parasites (hypnozoites) to prevent recurrent infection of blood, called a relapse
Gametocytocide	Drugs aimed at sexual blood-stage parasites to prevent infection of mosquitoes
Sporontocide	Drug aimed at stages of sporogonic development in the mosquito, including sporozoites
Radical cure	Elimination of clinically relevant forms of the parasite from the body using one or more drugs
Suppressive prophylaxis	Chemical suppression of patent parasitemia by a drug or drugs that are active against asexual blood stages
Causal prophylaxis	Prevention of infection of blood with a drug or drugs that are active against asexual liver stages
Presumptive antirelapse therapy	Presumptive treatment with a hypnozoitocide following exposure to infection, also called terminal prophylaxis
Relapse	New parasitemia originating from hypnozoites
Reinfection	New parasitemia originating from new infectious mosquito bite
Recrudescence	New parasitemia originating from the original parasitemia
Recurrence	New parasitemia of unknown origin

discovered resochin among patent archives registered by Winthrop Chemical Company in New York, NY, cartel partner to the German manufacturer (Bayer, then a subsidiary of IG Farben). Both drugs went to rushed wartime clinical trials, and resochin, renamed chloroquine by the Americans, emerged as the superior drug (50).

The recommended adult treatment was 25 mg/kg body weight administered in 3 doses over 48 h (typically 10 plus 10 plus 5 mg/kg at 24-h intervals). A 60-kg adult would thus consume a total dose of 1.5 g chloroquine base. However, this total dose far exceeded the minimal effective dose against *P. vivax* at that time. A series of experiments decisively demonstrated that total adult doses as low as 0.3 g consistently cured parasitemias of vivax malaria (27, 238). The developers of chloroquine nonetheless recommended the same dose for all malarias.

Chloroquine was, in almost all ways, the perfect drug for treating acute malaria in endemic settings. It was cheap, universally effective against all plasmodia species, deliverable over a brief period in few doses, and safe even for pregnant women and small children and came with few side effects. No drug like this had appeared before, and none like it has appeared since with these uniformly superior characteristics for a drug put to work in areas where medical supervision rarely occurs (60). Whenever and wherever resistance to chloroquine prompts its withdrawal from service, the drug that replaces it will be more expensive and more difficult to administer, the safety for pregnant women and small children will be less certain, and it may cause more marked side effects. No drug now available equals the overall effectiveness of chloroquine in the heyday of its now nearly vanished efficacy. The artemisinins may eventually rival chloroquine in this regard but only after conclusive demonstrations of safety for pregnant women and after market competition and industrial innovation drive down the cost and complexity of dosing.

Historically, chloroquine clears fever and parasitemia caused by *P. vivax* within, at most, 72 h of the first dose. The drug is very rapidly absorbed and slowly eliminated, principally as a parent drug and a desethyl metabolite in roughly 3:1 proportions (143). The plasma half-life is about 50 h, and therapeutic levels against vivax malaria persist in blood until about days 21 to 35 after the start of treatment (46, 132). In contrast, quinine is almost completely eliminated within 24 h of dosing (239). This difference in these two drugs has important implications for an understanding of the assessment of the therapeutic efficacies of both chloroquine and primaquine.

Figure 3 illustrates recurrent parasitemia after chloroquine or quinine therapy among subjects with vivax malaria acquired in 1940s tropical Asia or experimentally induced by challenge with sporozoites of the Chesson strain of *P. vivax* from New Guinea (18). Efficacy against blood stages has no bearing upon the difference in the shapes of these two curves. Instead, the pharmacokinetics of each respective drug and the impact upon relapse shape these curves. The postquinine recurrent parasitemias are known to be relapses because the same doses administered to subjects challenged with blood stages rather than sporozoites showed no recurrences (239). Whereas rapidly eliminated quinine allows essentially unfettered relapse, slowly excreted chloroquine eliminates new parasites emerging from the liver until, at about day 35, it finally falls below

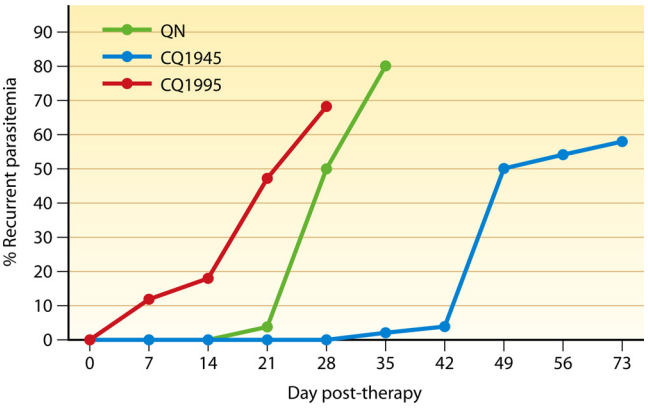

FIG. 3. Deterioration of chloroquine (CQ) efficacy between 1945 (green line) and 1995 (red line) among tropical Asian strains of *P. vivax* relative to natural relapse of the same strains following quinine (QN) therapy (blue line). The suppression of relapse by chloroquine in 1945 is due to lingering levels of drug in blood up to day 35, and the 1995 data suggest not only failure to suppress relapse but also failure to clear primary asexual parasitemia. Data were derived from various sources (12, 18, 47, 210, 239).

therapeutic levels, and emerging parasites survive to manifest patent parasitemia. The slow excretion and persistent activity of chloroquine in the blood represent the primary analytical tool for the classification of infections as being chloroquine sensitive or resistant, as shown by the 1995 postchloroquine recurrences in Fig. 3 (see Resistance to Chloroquine below).

Primaquine Therapy

Pamaquine (also called plasmochin) was an 8-aminoquinoline with the curious property of preventing the as-yet-not-understood process of relapse when discovered in the Elberfeld laboratories of Bayer in the 1920s in Germany (92). The drug proved to be too toxic for clinical use in humans, and only limited commercial distribution occurred. The strategic urgencies of the Second World War sparked a vigorous effort to discover a less toxic replacement. Primaquine, another 8-aminoquinoline, emerged from the same war-spurred program as chloroquine but was identified through a systematic survey of many hundreds of 8-aminoquinolines in birds and nonhuman primates. Eighteen candidates advanced to clinical trials. All of them seemed to be effective against relapse, but 8-aminoquinoline toxicity limited therapeutic choices (4). Primaquine had the highest therapeutic index and moved to licensure, production, and rushed use for American troops at war on the Korean Peninsula in 1950 (32).

In the earliest clinical trials of primaquine, investigators noted hemolytic anemia among African American subjects (65, 106) and ultimately discovered glucose-6-phosphate dehydrogenase (G6PD) deficiency (37), an inherited blood disorder linked to a survival advantage against falciparum malaria (190). Occurring at the q28 locus on the X chromosome, about 200 variant alleles have been described, with residual enzyme activities ranging from 0% to almost 100% (28). Heterozygous females often have mixed populations of normal and deficient red blood cells. The clinical consequences of G6PD deficiency and drug-induced hemolytic anemia range from mild and tran-

sient to severe and life-threatening. In general, residual enzyme activity correlates with the severity of reactions to hemolytic drugs (29). Among African A^- variants, primaquine-induced hemolysis tends to be mild and self-limited, apparently capable of destroying only senescent red blood cells. A^- G6PD-deficient subjects given primaquine relatively quickly recovered normal hematocrit levels, even in the face of continued standard therapy of 15 mg daily (7). The 45-mg weekly dose over 8 weeks was developed for safe and tolerable use in A^- G6PD-deficient patients (200), but the safety of this regimen among the many other variants has not been established and is rarely recommended.

Primaquine usually comes available as tablets containing 15 mg base. Most manufactured tablets cannot be reliably cut, and clinical literature on the drug typically refers to adult doses in 15-mg increments, i.e., 15- or 30-mg daily doses or 45-mg weekly doses (105). Studies by Schwartz et al. (200) and by Duarte et al. (70) demonstrated a higher risk of failure among heavier patients given the standard 15-mg daily adult dose for 14 days. An administration of a total dose of >3.5 mg/kg (or 0.25 mg base/kg body weight daily for 14 days) for small children and heavy adults was thus recommended. Most authorities now recommend 0.5 mg/kg daily for 14 days (105). In practice, however, the practitioner must choose between daily doses of 15 mg, 30 mg, and 45 mg among patients of most sizes. Doses of 15 mg for patients weighing 20 kg to 40 kg, 30 mg for those weighing between 41 kg and 70 kg, and 45 mg for those weighing >70 kg delivered doses ranging from 0.37 to 0.75 mg/kg up to 100 kg body weight. Patients weighing <20 kg often receive dosing approximated by manual crushing of tablets and delivery in liquid. Dosing in this review adheres to that predominating in the literature, with reference to the daily dose in milligrams base delivered to an adult weighing 41 to 70 kg.

The standard adult regimen of primaquine for the prevention of relapse was, until recently, 15 mg daily for 14 days. Some alleged that this regimen reflected the shipboard transit time from Korea to the United States for returning U.S. soldiers. However, the more likely genesis of this relatively inconvenient regimen may be found in the tolerance studies done by Clayman and colleagues using prison inmate volunteers (42). They administered single doses of primaquine ranging from 5 to 215 mg to fasted subjects and measured the frequency and intensity of gastrointestinal upset. Almost none complained with the 5-mg dose, and the 215-mg dose caused "immediate and severe cramping." The 15-mg dose prompted complaints from only 10% of subjects, whereas the 30-mg dose caused 30% of subjects to complain. The idea that exceeding the 15-mg dose caused unacceptable intolerance and toxicity took hold among malariologists and severely limited the useful application of this otherwise extremely versatile drug. The view on primaquine toxicity expressed by the prominent malariologist David F. Clyde (45) represented the broader-held viewpoint that, "… the fact that the therapeutic dose and the toxic dose are close leads to serious problems associated with its use."

During the early 1990s, an effort to develop an in vivo test procedure for chloroquine-resistant *P. vivax* led to experimentation with primaquine as a means to suppress the confounding of that test by reinfection and relapse. A clinical trial of primaquine for this purpose applied 30 mg every other day, and it was found to be as well tolerated as chloroquine prophylaxis (13). Those authors cited the seminal paper on primaquine tolerability by Clayman et al. (42) and described a rarely cited aspect of that early study: when primaquine was administered with food (rather than fasted), the prisoner volunteers who took "even the highest doses" of primaquine (presumably 215 mg) registered no complaints. Food greatly improved the tolerability of primaquine and emboldened subsequent randomized, double-blind, placebo-controlled trials of 30 mg primaquine daily lasting from 11 to 50 weeks (16, 79, 234). In those trials, primaquine was as well tolerated as placebo. These studies in turn likely emboldened authorities to recommend 30 mg daily for 14 days as the standard adult dose for primaquine therapy.

Primaquine therapy against liver stages exhibits an unusual and poorly understood phenomenon referred to as the total dose effect. In both preclinical and clinical trials, the total dose of primaquine seemed to be the key determinant of efficacy regardless of the duration over which the dose was administered. In 1977, Schmidt et al. (197) carefully described the effect using rhesus monkeys infected by *Plasmodium cynomolgi* (a *P. vivax*-like parasite with hypnozoites and relapse behaviors). In their experiments, they administered a therapeutic dose of primaquine with an effective companion drug (chloroquine or quinine) as a single dose or as daily doses administered over 3, 7, or 14 days: all showed equally good efficacies against relapse. Clinical trials demonstrated the same phenomenon at work with vivax malaria. In 1977, Clyde and McCarthy (44) described 11 human subjects experimentally challenged with *P. vivax* Chesson and given 30 mg daily for 7 days instead of 14 days, and none relapsed. The weekly administration of 45 mg primaquine for 8 weeks with good efficacy against relapse demonstrates the total dose effect even more dramatically (7). The perception of toxicity and poor tolerability for higher daily doses (>15 mg) of primaquine limited the exploitation of such versatility. Indeed, Clyde and McCarthy (44) concluded their abstract with, "However, it is stressed that because of the risk of primaquine-induced hemolysis in individuals having genetically transmitted erythrocyte abnormalities this high dosage [30-mg daily adult dose] should not be used routinely." Such viewpoints suppressed the opportunity to explore more practical (higher-dose, shorter-duration) regimens of primaquine.

The 14-day dosing regimen of primaquine almost certainly diminishes its effectiveness. In many countries in zones of endemicity, a 5-day regimen (15 mg daily) of primaquine was adopted and used for decades both on the basis of evidence from poorly controlled clinical trials and in an effort to improve effectiveness (19). That regimen was later proven to lack efficacy, and the practice has been largely abandoned. The safety, tolerability, and efficacy of primaquine administered in twice-daily doses of 60 mg base over 3 days should be explored for use in people with access to screening for pregnancy and G6PD deficiency (17).

Primaquine may not be effective against liver stages unless it is administered with another drug. As early as 1955, Alving and colleagues (6) asserted this with a standard 15-mg daily dose (14 days) against *P. vivax* Chesson, with one treatment group receiving quinine therapy before starting primaquine therapy and the other group receiving the two therapies concurrently.

The relapse rates among these two groups of 19 human subjects each after 6 to 12 months of follow-up were 95% and 21%, respectively. Edgecomb et al. (73) did this experiment using the 30-mg daily dose without and with concurrent quinine and reported relapse rates of 80% and 3%, respectively. Essentially similar findings were made in clinical trials of pamaquine and quinine during the 1940s. Berliner and colleagues (26) found that 90 mg pamaquine (daily for 14 days) given concurrently with standard quinine therapy provided 100% efficacy against relapse of the Chesson strain, but when the same regimen was applied serially (quinine followed by pamaquine), all subjects relapsed. Craige et al. (62) saw a similar effect when applying a less efficacious 63-mg daily pamaquine regimen against the Chesson strain. According to Schmidt (195), challenge studies done by the U.S. National Institutes of Health at the Stateville Penitentiary in 1949 applied pentaquine in combination with quinine and achieved cure for 8 of 11 subjects. However, when the same dose of pentaquine was administered with chlorguanide rather than quinine, only 1 in 10 subjects was cured. Schmidt (195, 196) found essentially similar findings with *Plasmodium cynomolgi* infection of rhesus macaques. These experimental outcomes, most reported over 50 years ago, elicit incredulity today. Primaquine monotherapy has been viewed as being effective against hypnozoites because the practical importance of all of these studies was forgotten with the deployment of primaquine and chloroquine as first-line, companion therapies for the radical cure of vivax malaria.

These studies are conspicuously relevant today. New blood schizontocides supplanting chloroquine as frontline therapy for acute vivax malaria would thus represent new companion drugs for primaquine. The same is true for new drugs aimed at hypnozoites, especially the 8-aminoquinoline candidates. These studies illustrate the necessity of gauging the impact of companion blood schizontocide upon the efficacy of the hypnozoitocide against relapse.

Experimental Therapies

Drug development strategy. The availability of blood schizontocides for vivax malaria has been dependent upon drugs developed for falciparum malaria. Drugs in advanced development, or even postmarketing, are typically then applied to vivax malaria in either nonhuman primate studies or in clinical trials. This approach presents obvious advantages, especially in the vital stretch of early drug development that includes in vitro screening of candidate drugs for efficacy against blood stages. A similar high-throughput screening specific for vivax malaria remains impractical in the absence of a system of continuous in vitro cultivation. Fortunately, the pipeline for blood schizontocides against falciparum malaria has rarely failed with vivax malaria. Almost every drug licensed for falciparum malaria in the past 60 years has also proven to be safe and effective for the treatment of acute vivax malaria, with the exception of sulfadoxine plus pyrimethamine (SP) (widely marketed as Fansidar). Even that exception may have been a misperception (see Resistance to Antifolates below). Chloroquine, mefloquine, halofantrine, piperaquine, and the artemisinins all proved to be effective against acute vivax malaria. Until *P. vivax* becomes successfully adapted to continuous in vitro cultivation, this shared approach of drug discovery will be applied.

The early work of Alving and colleagues (6, 26, 62, 73) showed that primaquine apparently required an appropriate companion drug to effect the elimination of hypnozoites. This consideration adds substantial technical complexity to the development of therapies for vivax malaria. The development of blood and tissue schizontocides/hypnozoitocides within independent tracks has been the dominant approach. The isolation of these therapeutic targets appeals to technical sensibility, but it may also impose unappreciated hazards. Should the interpretation of the data reported by Alving and colleagues (6, 26, 62, 73) prove true, the segregated development of blood and tissue schizontocides would risk fielding blood schizontocides that leave primaquine (or another hypnozoitocide) impotent against hypnozoites. Likewise, that approach imposes a risk of losing entire families of drugs against hypnozoites by failing to screen candidates with companion blood schizontocides. Had the discoverers of primaquine not used quinine or chloroquine in their trials, the drug would very likely have been discarded and lost. A direct linkage of the development of blood and tissue therapies against vivax malaria may be required to avoid these risks. Coping with that development strategy requires overcoming technical, logistical, and regulatory challenges.

Hypnozoitocides. The neglect of clinical trials evaluating hypnozoitocidal drugs very likely reflects the relative difficulty of such work. The rational analysis of such trials requires both a relative abundance of naturally infected study subjects and their willingness or availability for long-term follow-up and without risk of reinfection. This last requirement rules out an area of endemicity, whereas the first requirement demands an area of endemicity, a formula of mutual exclusion leaving few opportunities for meaningful work. Nonetheless, such studies are possible with travelers who acquire vivax malaria and return to a nonendemic area. Soldiers, migrant workers, or tourists may serve as likely study populations in urban research centers. Mahidol University's Hospital for Tropical Diseases in Bangkok, Thailand, has employed this strategy, and its productivity in clinical research of 8-aminoquinolines against liver stages demonstrates feasibility. Nonetheless, few centers engage in such research because its vital importance to patient and public health has not been adequately appreciated. The experimental challenge data from the clinical screening of 8-aminoquinolines during the 1940s and 1950s show decisively that follow-up to only 28 days is not adequate for assessing clinical efficacy against relapse: more than half of drug failures occurred beyond day 28 (4).

A notable exception to the broad neglect of hypnozoitocides has been the development of tafenoquine as the intended successor to primaquine for the prevention of relapse (165). This drug was invented by the U.S. Army and later developed in clinical trials by GlaxoSmithKline (United Kingdom) and, later still, by the Medicines for Malaria Venture (a consortium of private and public agencies). The developmental path of this drug, more tortuous than most, included an initial indication for prophylaxis only (32). It is currently being developed as an antirelapse drug (232). Tafenoquine exerts an extraordinarily broad spectrum of activity against blood and tissue stages of *P. falciparum* and *P. vivax*, killing liver-stage schizonts and hyp-

TABLE 3. Therapies for acute vivax malaria with and without primaquine[a]

Therapy	Location	No. of subjects	Randomized	% Efficacy		Reference
				Day 7	Day 28	
Without primaquine						
SP+AS	Afghanistan	90	Yes	100	≥96	121
DH+PP	Indonesia	90	Yes	99	98	179
LF+AR	Indonesia	85	Yes	99	89	179
AQ+SP[b]	PNG	98	Yes		89	142
SP+AS[b]	Indonesia	19	Yes	100	100	223
With primaquine						
AS	Vietnam	28	No	100	96	64
AS	Thailand	299	Yes	100	100	203
RF	Thailand	5	Yes	0	0	173
MQ	Indonesia	295	Yes	100	100	138
AZ	India	97	Yes	84	88	71
DH+PP[c]	Indonesia	54	Yes	98	96	99
AQ+AS[c]	Indonesia	60	Yes	97	92	99
DH+PP[c]	Indonesia	83	Yes	100	100	171
Without primaquine						
AS	Thailand	316	Yes		50	203
AS	Thailand	20	Yes	100	40	175
AR	Thailand	20	Yes	100	48	175
HF	Thailand	23	Yes	100	40	175
QN	Thailand	22	Yes	100	48	175
PQ	Thailand	30	Yes	100	96	175
CQ	Thailand	30	Yes	100	100	175
MQ	Thailand	20	Yes	100	100	175
SP	Thailand	12	Yes	56	28	175
TC	Thailand	18	Yes	100	78	176
DX	Thailand	18	Yes	100	57	176
CL	Thailand	12	Yes	100	58	176
AZ	Thailand	18	Yes	100	20	176

[a] AS, artesunate; DH, dihydrartemisinin; PP, piperaquine; LT, lumefantrine; AR, artemether; AQ, amodiaquine; PQ, primaquine; RF, rifampin; MQ, mefloquine; AZ, azithromycin; HF, halofantrine; QN, quinine; CQ, chloroquine; TC, tetracycline; DX, doxycycline; CL, clindamycin.
[b] Administration or withholding of primaquine therapy not explicitly detailed.
[c] Unsupervised primaquine offered to study subjects after completing these therapies.

nozoites, sexual and asexual blood stages, and stages in the mosquito host (52, 157, 164, 169). The long plasma half-life of this 8-aminoquinoline (14 days) contrasts with the very rapid elimination of primaquine (half-life of 4 h) and provides incidental prophylactic protection lasting for several months (201). Its utility as a potential tool for malaria elimination should be conspicuous: in concept, a single course of therapy could eliminate disease, relapse, and further transmission. Moreover, this treatment could eventually eliminate the infection of mosquitoes by virtue of a period of effective prophylaxis extending beyond the lifetime of most anopheline mosquitoes. Perhaps the greatest challenge with tafenoquine for any broad therapeutic application is the likelihood of hemolytic toxicity in G6PD-deficient people, a safety challenge exacerbated by its very long plasma half-life.

Another experimental therapy, elubaquine (formerly known as bulaquine), has shown promise as a hypnozoitocide (72). Elubaquine is a prodrug of primaquine invented at the Central Drug Research Institute, India. It is rapidly metabolized to primaquine and exhibits most of primaquine's pharmacokinetic characteristics (145). The two drugs showed similar efficacies against relapse in a limited number of trials, each with inherent limitations. In a study in Thailand (126), follow-up occurred only to day 28, and a study in India (1) applied a suboptimal 5-day regimen. Nonetheless, in these limited studies, elubaquine showed performance at least equal to that of primaquine in preventing relapse. Comparisons of safety proved to be much more interesting in the Thai study: among four subjects with G6PD deficiency treated with primaquine, all experienced significant drops in hematocrit levels (a mean of 39% on day 1 to a mean of 21% on day 8), whereas none of the three G6PD-deficient subjects treated with elubaquine did so (126). If elubaquine proves as efficacious as primaquine and yet somehow does not cause hemolytic anemia in G6PD-deficient subjects, its value in attacking endemic vivax malaria would be enormous.

Blood schizontocides. An assessment of experimental therapies for acute vivax malaria requires grappling with the ambiguities imposed by relapse. Table 3 represents a wide range of clinical data available on largely experimental therapies for acute vivax malaria. Studies are segregated into three broad categories according to the withholding of primaquine from combined therapeutics, its application with either mono- or combined therapies, or its withholding from monotherapies (Table 3). Cure rates are given for day 7 and day 28 following the start of therapy, and the reason for doing so should be obvious by an examination of outcomes in Table 3. Drugs such as artesunate, artemether, halofantrine, and quinine show excellent efficacy at day 7 but uniformly poor efficacy at day 28. In contrast, primaquine, chloroquine, piperaquine, and meflo-

quine show almost complete efficacy at both points. With the notable exception of primaquine, what separates these classes of outcomes is the plasma half-life of the therapeutic applied. Relapses after the rapid loss of therapeutic levels of drug appear to account for the very poor cure rates at day 28. Likewise, the excellent cure rates with chloroquine and mefloquine appear to be the product of a lengthy plasma half-life and the successful suppression of relapse. The outcomes with primaquine tend to affirm this interpretation: it is very rapidly eliminated and yet showed an excellent cure rate at day 28, and its activity against relapse seems to be the most likely explanation. These data also illustrate the very high risk of relapse among subjects infected by *P. vivax* in Thailand: apparently >50% within 28 days of patency.

An assessment of experimental therapies in such clinical trials thus requires a grasp of at least three important variables: (i) the plasma half-life of the therapy applied, (ii) the presence or absence of primaquine with therapy, and (iii) the geographic origin of the infection being treated (risk of relapse within the duration of evaluation). What appear to be poor therapeutic blood schizontocidal outcomes may be entirely attributable to relapse, as suggested by the data summarized in Table 3. On the other hand, a very poor outcome against asexual blood stages may be masked by relapses that are neither eliminated (by primaquine) nor suppressed (by lengthy plasma half-life therapies). This ambiguity limits the utility of clinical trials compartmentalized for the examination of blood-stage infection only.

The data from Indonesia, listed in Table 3 (179), illustrate this quandary. Those authors examined the effectivenesses of both dihydroartemisinin plus piperaquine (DH+PP) and lumefantrine plus artemether (LF+AR) against acute vivax malaria among residents of southern Papua. Both drugs showed very good efficacy at day 7. At day 28, 98% of subjects taking DH+PP remained free of parasites, whereas this was true for only 89% of those given LF+AR. Concluding that DH+PP was the superior blood schizontocide would be subject to error. The difference in these therapies may be the product of successfully suppressed relapse by a relatively longer persistence of effective levels of piperaquine in blood than lumefantrine. Both drugs may have effected a complete blood-stage cure, but one more successfully suppressed relapse than the other, and the suppression of relapse was not the clinical objective of either therapy. On the other hand, one cannot rule out therapeutic failures lurking among the late recurrences of parasitemia following LF+AR treatment. In the absence of a means of distinguishing relapse from reinfection or recrudescence, identification of the superior blood schizontocidal therapy remains elusive.

The inclusion of primaquine with the blood schizontocide being evaluated partially addresses the problem of confounding by relapse, but it also introduces another confounding factor. With the exceptions of rifampin (203) and azithromycin (173), the listed therapies showed very good estimated efficacies against blood stages (Table 3). With the possible exception of Thailand, however, few other areas can offer data supporting the assumption of complete efficacy against relapse by *P. vivax* with any given regimen of primaquine. Moreover, primaquine itself exerts potent blood schizontocidal activity against *P. vivax* (174, 175, 236). The inclusion of primaquine therapy in evaluations of therapies aimed at asexual blood stages introduces the possibility that the observed good efficacy may not be fully attributable to the blood schizontocide under evaluation.

Resolving these ambiguities with blood-stage therapies for acute vivax malaria must await a technology that reliably discriminates relapse from reinfection or recrudescence as the source of recurrent parasitemia after therapy. Alternatively, a distinct approach to clinical trials for vivax therapies may be applied. Rather than attempting a dissection of blood- from tissue-stage therapeutic effects, one may simply accept radical cure as the sole therapeutic objective. The evaluation of therapies against vivax malaria for real-world use should be combined for the elimination of blood- and tissue-stage parasites. Any recurrent parasitemia not attributable to reinfection may be considered to be a therapeutic failure without regard to which particular compartment failed.

In assessing the efficacy of radical cure of vivax malaria, the period of posttherapy follow-up matters a great deal. The data in Table 3 show a good efficacy of applied therapies as a radical cure, at least within the 28 days of observation. The importance of considering the follow-up period may be seen in the data for mefloquine shown in Table 3 (173, 175). With or without primaquine, the suppression of early relapse may create the illusion of effective radical cure. This will be true of any drug with a similarly long plasma half-life. Thus, detecting relapse due to primaquine failure in radical cure requires addressing adequate follow-up with either no risk of reinfection or ascertained rates of reinfection for attributable risk estimations.

The ideal randomized, controlled trial of a therapy for radical cure would measure recurrent parasitemia among subjects not exposed to a risk of reinfection during a 1-year follow-up. The standard and experimental therapy groups would each be composed of sets of drugs designed to eliminate asexual blood and liver stages. The study would be powered to demonstrate the noninferiority of the experimental therapy with respect to safety and efficacy. The cumulative incidence of recurrent parasitemia over the course of 1 year provides the basis of comparison. The same trial in an area of endemicity (and having a risk of reinfection during follow-up) would require two additional treatment arms: one to measure the incidence of reinfection (effective radical cure control) and the other to measure the incidence of new parasitemia due to relapse (effective blood-stage cure only).

All of the analytical issues detailed here also apply to assessments of standard therapies against vivax malaria, as in gauging resistance. However, the practical option of not segregating blood- from tissue-stage activities cannot be applied in the instance of demonstrating biological resistance to standard therapy. A liver-stage parasite resistant to primaquine may be wholly sensitive to chloroquine in the blood stage. These effects must be segregated in order to develop practical therapeutic strategies for coping with resistance. A demonstration of stage-specific resistance represents the analytical cornerstone of an understanding of the problem.

RESISTANCE TO CHLOROQUINE

Definition

Asexual blood-stage parasites that have been demonstrated to survive a normally absorbed standard regimen of therapy may be classified as being resistant to that therapy. In a pharmacokinetic sense, one typically gauges resistance against either peak levels of drug during the course of treatment or the area under the curve of bioavailable concentrations of drug. For malaria, this means identifying recurrent parasitemia as a recrudescence, but such a perspective in defining the resistance of *P. vivax* to chloroquine has proven elusive. The only published methodology detailing a means of diagnosing resistance to chloroquine with this parasite (14) carries ambiguities imposed by the phenomenon of relapse and, to a lesser extent, reinfection.

In the case of *P. falciparum*, recurrent parasitemia following therapy may be either a recrudescence or a reinfection. Although somewhat nuanced with respect to the possibility of coinfecting strains and PCR sensitivities, a relatively simple check of parasite genotypes allows classification of recurrent parasitemia as a recrudescence (matched genotype with primary parasitemia) or reinfection (mismatched genotype) (205). Recurrences classified as reinfections are typically discounted as losses to follow-up during the 14- to 42-day follow-up period (94).

In contrast, *P. vivax* hypnozoites essentially remove this tool of discernment from the in vivo test format. A perfectly matched genotype may be either a recrudescence or a clonal relapse (40). Likewise, mismatched primary and secondary parasite populations may represent either a new infection or a relapse (141). Putting aside the complex issue of inbreeding frequencies and clonal population structure (8), a single mosquito may carry sporozoites representing at least several genotypes from any number of successful gamete unions (185). Thus, a single brood of invading parasites may yield a primary tissue schizont and patent parasitemia of genotype A and a hypnozoite of genotype B, or the hypnozoite may be genotype A. Even discounting the more vexing problem of dealing with people with an accumulation of multiple broods of hypnozoites lying dormant in their livers (47), genotyping does not now allow discerning parasitemia originating from recrudescence, relapse, or reinfection.

The methodology for diagnosing resistance to chloroquine (14) dealt with this problem by sidestepping the necessity of distinguishing recrudescence from relapse or reinfection. The test hinges upon lingering levels of chloroquine in blood for 28 days following therapy and the minimally effective concentration of chloroquine and its major metabolite, desethylchloroquine (CQ+DCQ), in blood. By examining the graphs in Fig. 3, it may be appreciated that in the 1940s and 1950s, chloroquine prevented the patency of relapses up to day 35. The contrasting postquinine recurrences represent relapses and not recrudescences: the effective killing of blood stages by quinine was demonstrated by the absence of posttreatment recurrences among subjects challenged with blood stages rather than sporozoites (239). Chloroquine or quinine alone exerts no effect upon latent liver stages (239), and the differences in their respective excretion rates (slow versus rapid, respectively) account for the marked difference in the shapes of the posttherapy recurrence curves. One may thus be confident that the curve of recurrences after quinine treatment represents true relapse and that the suppression of relapse after chloroquine treatment represents the blood schizontocidal effect of lingering drug in the blood (14). If so, the onset of recurrences at about day 35 after chloroquine treatment should represent the threshold at which drug levels slip below the minimally effective concentration for sensitive parasites.

Several lines of evidence supported that supposition and helped to establish the estimated minimally effective concentration of CQ+DCQ in whole blood, the value that ultimately segregates sensitive from resistant phenotype classifications in the in vivo test format. First, Berliner et al. (27) measured the minimally effective concentration of chloroquine against *P. vivax* in the 1950s, presumably when resistant phenotypes were not more than very remotely likely to occur. Those authors measured it at 15 ng/ml plasma, which corresponds roughly to 120 ng/ml whole blood (by multiplying by a factor of 8, derived from several parallel measurements of the two compartments) (2, 183). Rombo et al. (184) approached the same measurement from the perspective of effective prophylaxis and derived a value of 90 ng/ml whole blood. Finally, CQ+DCQ following therapy show mean levels near 100 ng/ml at day 35, the point at which relapses of chloroquine-sensitive parasites begin breaking through. Taking into consideration all of these observations, the value of 100 ng/ml was selected as an estimate of the minimally effective concentration of CQ+DCQ in whole blood (14). Direct observation of this threshold should be confirmed by measuring CQ+DCQ levels at the onset of relapse following the treatment of chloroquine-sensitive vivax malaria.

The diagnosis of resistance to chloroquine thus involves the critical measurement of CQ+DCQ in blood on the day of recurrent parasitemia. Whether recurrent parasitemia represents recrudescence, relapse, or reinfection is not known, but we know that the parasite has broken through concentrations of drug in blood that ordinarily eliminate parasites of the chloroquine-sensitive phenotype. This currently stands as the only functional definition of resistance to chloroquine in vivax malaria. Other possible means of making the diagnosis (ex vivo and animal models) along with the nuanced interpretation of in vivo testing are detailed below.

Diagnosis

Several options exist for examining the response of *P. vivax* to chloroquine: the 28-day in vivo test, ex vivo assays, and animal model systems. Each of these is detailed here.

Twenty-eight-day in vivo test. The rationale for the 28-day in vivo test is detailed above. This section focuses on the conduct of the test, classification of therapeutic responses, and identification of pitfalls with the test.

Some standardized in vivo tests impose an unnecessarily stringent limit on parasite densities as an enrollment criterion. This creates a potentially powerful selection bias when one attempts to ascertain therapeutic responsiveness in communities by arbitrarily eliminating what is likely to be the median parasite density in the community. Investigators should qualify any subject having a microscopically proven diagnosis of *P.*

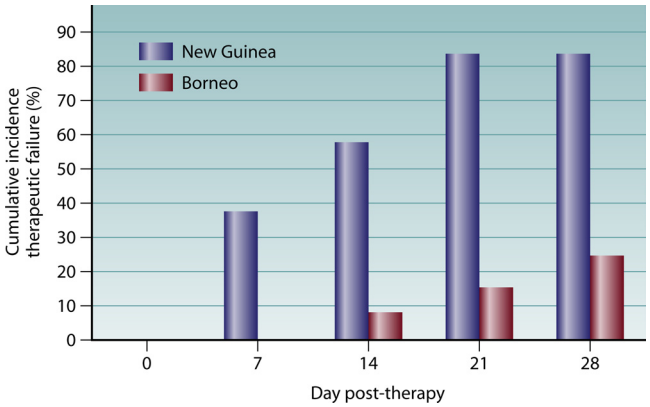

FIG. 4. Plot of the cumulative incidence of recurrent parasitemia after chloroquine therapy of vivax malaria in western Indonesian Borneo (167) and eastern Indonesian New Guinea (11).

vivax asexual forms for enrollment, although hyperparasitemia (not formally defined, but a value of >100,000 parasites/μl has been generally applied) should prompt intravenous quinine therapy and thus disqualify the patient as a study subject. Prior antimalarial therapy should not disqualify a potential subject, as this tends to be the rule in areas of endemicity. Excluding such subjects may also introduce selection bias in the estimate of community failure rates.

Administration of therapy must exclude a primaquine adjunct until after day 28 because primaquine itself effectively clears blood-stage parasites. The inclusion of primaquine at the start of the test may therefore mask chloroquine-resistant infections and cause an underestimation of the risk of therapeutic failure with chloroquine (14). Chloroquine therapy should be directly observed by the research or clinical team or by a responsible family member. The subject should be monitored with blood film examinations by a clinically responsible and practical schedule to day 28.

A subject with an asexual parasitemia level that either increases or remains stable (≥25% of day 0 parasitemia) to day 2 should be given alternative therapy and provisionally classified as a direct treatment failure. A subject having an asexual parasitemia of any level persisting to day 7 should be given alternative therapy and provisionally classified as an early treatment failure. For an asexual parasitemia that becomes undetectable but reappears at any time between days 7 and 28, the subject should be given alternative therapy and provisionally classified as a recurrence treatment failure. Subjects having no recurrent parasitemia up to day 28 should be classified as having had an infection that was sensitive to chloroquine.

Direct and early treatment failures may be definitively classified with a demonstration of adequately absorbed, fully compliant therapy. This may be done by a direct observation of all doses or by measuring CQ+DCQ levels after the last dose (>500 ng/ml whole blood is considered to be adequate). The definitive classification of resistant phenotypes among recurrence failures must await a measurement of CQ+DCQ in whole blood at the time of recurrent parasitemia. When CQ+DCQ levels for any recurrence are below 100 ng/ml, the infection must be classified as being ambiguous with respect to sensitivity to chloroquine. The subject is considered to be a loss to follow-up when later calculating the risk of therapeutic failure in the community. When CQ+DCQ levels exceed 100 ng/ml, the parasite in that subject may be classified as being resistant without regard to which day the parasite appeared.

Individual classifications of direct, early, or recurrent treatment failures provide a generally useful empirical sketch of the relative level of resistance in the community. This approach also permits assigning relative levels of resistance to individual parasite isolates. However, the best quantitative approach to the characterization of measuring the adequacy of chloroquine against vivax malaria in communities is the calculation of the cumulative incidence of therapeutic failure across weekly intervals using the life table method (14). The life table method manages the probabilistic impact of losses to follow-up due to the inability to find the subject, intercurrent parasitemia by another species, or reappearance of vivax malaria with a value of <100 ng/ml (almost all of which invariably occur). The interval-specific cumulative incidence (or risk) of therapeutic failure constitutes the basis for the data points shown in Fig. 4, and Table 4 demonstrates the calculation of cumulative incidence by the life table method. If the timing of recurrence correlates with the degree of resistance (in correspondence with levels of drug penetrated), a plot of 7-, 14-, 21-, and 28-day cumulative incidences should intuitively reveal such distributions in any community surveyed.

The classification of parasite isolates as being sensitive or resistant to chloroquine requires caution, especially when these phenotypes are subsequently applied to studies of genetic characteristics possibly linked to drug resistance. Parasites appearing on day 0 and failing to reappear within 28 days may be confidently classified as being sensitive. The same may be said of direct and early treatment failures. However, given the ambiguities inherent with recurrent parasitemia during the 28-day test, the drug resistance phenotype of parasites collected on day 0 remains uncertain. The parasites on day 0 that are ultimately cleared may have been exquisitely sensitive to chloroquine, and the parasites that later appeared during the

TABLE 4. Life table estimation of cumulative incidence (risk) of recurrent parasitemia after chloroquine therapy of vivax malaria[a]

Day	No. of subjects remaining at risk	No. of cases of therapeutic failure	No. of subjects withdrawn for any reason	Interval risk[b]	Cumulative risk[c]
0	34	0	0	0	0
2	34	2	1	0.0597	0.0597
4	31	0	0	0	0.0597
7	31	3	1	0.0984	0.1522
11	27	5	2	0.1923	0.3152
14	20	4	0	0.2000	0.4522
18	16	2	0	0.1250	0.5207
21	14	0	1	0	0.5207
28	13	3	2	0.2500	0.6405

[a] Reprinted from reference 15 with permission of the publisher.

[b] Interval risk was calculated as follows: $i = [N - (w/2)]^{-1}$, where N is the number of subjects remaining at risk, i is the number of cases of therapeutic failure, and w is the number of subjects withdrawn for any reason.

[c] The cumulative incidence of therapeutic failure (CR_n) was calculated as follows: $CR_n = 1 - [(1 - IR_n) \times (1 - CF_{n-1})]$, where IR is interval risk, n is the day of the test, and $n - 1$ is the prior interval. To calculate the CR for day 11, first estimate the interval risk as follows: $5[27 - (2/2)]^{-1} = 0.1923$ and, finally, $1 - [(1 - 0.1923) \times (1 - 0.1522) = 0.3152$. Thus, the 11-day cumulative incidence (or risk) of therapeutic failure was 31.5%.

test could represent a relapse or reinfection by a chloroquine-resistant strain. Therefore, only the parasites appearing on the day of recurrence may be unambiguously classified as being resistant to chloroquine.

The most important limitation of this in vivo test for chloroquine is its practical irrelevance to any other drug. The conduct and interpretation of the test rest on the highly specific foundation of chloroquine excretion rates and minimally effective concentration in blood. Any other drug would impose fundamentally different rules of logic necessarily supported by a detailed knowledge of pharmacokinetics and parasite sensitivities to the drug. Another obvious limitation of the in vivo test is the need to have patients at all and then to be burdened with monitoring them for 4 weeks. Also, the requirement for measuring drug levels in blood severely limits the number of clinics or laboratories that can do reliable testing (employing rigorous high-pressure liquid chromatography of drug/metabolite extracted from volumetric blood dried onto filter paper). Finally, confounding by natural immunity may be an important limitation, especially in regions of high endemicity.

Ex vivo assay systems. The term ex vivo distinguishes systems using parasites removed from a subject and studied over a limited period of time from the in vitro systems that use preserved or continuously maintained parasites that may be studied indefinitely in those systems. Limited successes have been achieved with attempts to establish *P. vivax* in in vitro systems. To date, there is no practical means of doing so. In contrast, a variety of ex vivo techniques have been developed for the direct observation of *P. vivax* in simplified laboratory systems. Most of these techniques involve evaluating the inhibitory effect of blood schizontocides on live *P. vivax* asexual blood stages (43, 84, 217). Relative objectivity represents the principal advantage of ex vivo assays of drug sensitivity. In the ideal system, parasites attempt growth in the absence of potentially powerful confounding effects like host immunity, compliance to prescribed therapy, absorption of drug, and the above-discussed ambiguities regarding the origin of recurrent parasitemia. Moreover, as described below, ex vivo systems provide vital information on mechanisms of drug activity against parasites and allow an exploration of the genetic bases of drug-resistant phenotypes.

A detailed consideration of all of the published work in this field exceeds the limits of this review. The focus here will be on recent work from Thailand, Indonesia, and Myanmar highlighting two distinct approaches to the problem of gauging parasite sensitivity to antimalarials.

Druilhe and colleagues (69) examined 17 isolates from an area in southern Myanmar having documented resistance to chloroquine in *P. vivax* with a cohort of subjects taking chloroquine prophylaxis. Those researchers employed antiparasite lactate dehydrogenase monoclonal antibodies to capture the enzyme for detection by enzyme-linked immunosorbent assay, with optical density output representing a measure of protein synthesis. They employed a 50% inhibitory concentration cutoff for the sensitivity of 100 nM chloroquine described by others working with presumably chloroquine-sensitive *P. vivax* isolates from Thailand (59, 218) and found 2 of the 17 isolates to be resistant in their system. The primary advantage of the lactate dehydrogenase capture system may be the objective readout of optical density in an enzyme-linked immunosorbent assay platform and its apparently superior sensitivity relative to that of [^{3}H]hypoxanthine incorporation (measuring DNA synthesis) or microscopic examination.

Recent work from a research group of Thai, Indonesian, Australian, and British collaborators dissected the key determinants of ex vivo assay systems for *P. vivax* (189, 202). These investigators derived a 50% inhibitory concentration threshold for sensitivity based upon the known level of clinical resistance of 65% at their study site (Timika, Papua, Indonesia). In other words, they drew the ex vivo threshold where it classified 65% of isolates as being resistant: 220 nM chloroquine. The basis for the distinction between this estimate and the 100 nM value described by Congpuong et al. (59) may lie in subsequent findings regarding the developmental stage specificity of chloroquine in *P. vivax*. Sharrock et al. (202) and Russell et al. (189) identified important determinants of ex vivo responsiveness that would have been missed in more-automated systems. They found *P. vivax* late trophozoites to be insensitive to chloroquine, in sharp contrast with *P. falciparum*, and this key discovery profoundly impacts the rationale and interpretation of ex vivo assessments.

Parasitemia of *P. vivax* in patients often presents with most stages of asexual development detectable in smears of peripheral blood. On the other hand, peripheral blood collected from patients with *P. falciparum* usually contains parasitemias only at the early trophozoite (ring) stage of development; more-mature asexual forms tend to sequester in the deep vasculature. In working with *P. vivax* ex vivo, the specificity of chloroquine against early trophozoites emerges as a potentially powerful confounding factor. If chloroquine fails to attack more-mature trophozoites, their relative contribution to the population going into the ex vivo well will obviously impact maturation end points. A relatively mature parasitemia would appear to be, perhaps falsely, highly resistant to chloroquine. Apart from the practical importance of this finding, it also sharply highlights the conspicuous lack of even the most fundamental understanding of how chloroquine kills *P. vivax*. We have not known the stage of parasite targeted by chloroquine, much less its mechanism of activity. The finding of chloroquine-insensitive trophozoites in *P. vivax* suggests a mechanism of activity wholly distinct from that implicated for *P. falciparum* and linked to hemozoin synthesis (detoxification of digested hemoglobin heme by chemical aggregation). Visible clumps of hemozoin appear in young ring forms of *P. falciparum*, but in *P. vivax*, these do not appear until the blood-stage schizont reaches maturity. Thus, late hemozoin synthesis in *P. vivax* appears to be unaffected by chloroquine.

The development of a standardized ex vivo assay for assessing the therapeutic susceptibility of discrete isolates of *P. vivax* should be a very high research priority. That effort must identify a means of analytically coping with the apparently inherent late-trophozoite-stage insensitivity to chloroquine and perhaps other drugs. Assumptions of similar mechanisms of blood schizontocidal activity between *P. falciparum* and *P. vivax* should be abandoned, and work should be focused upon elucidating mechanisms of activity in the treatment of vivax malaria.

Animal models. Nonhuman primate models have long provided access to well-characterized strains of *P. vivax* to laboratories located far from zones of endemicity. Much of the work focused upon preclinical drug and vaccine studies, especially

for *P. falciparum*. Similar work occurred with *P. vivax*, and the availability of animal models served the additional role of addressing relatively simple standing questions created by the inability to maintain the parasite under continuous in vitro cultivation. One of those was the question of resistance to chloroquine in *P. vivax*. In the absence of an in vitro assay and of molecular evidence of resistance, could the relatively ambiguous clinical failures observed for patients in areas of endemicity be confirmed by more-objective means?

P. vivax readily infects a number of other primates including chimpanzee, gibbon, owl monkey, squirrel monkey, spider monkey, some tamarins, and white-faced monkey (51). The owl and squirrel monkeys have emerged as the favored model by virtue of availability and ease of care and infection (54). Infections by chloroquine-sensitive strains of *P. vivax* have been treated using these models, including the Chesson, Palo Alto, and Achiote strains (58, 186, 194). This record of sensitivity of strains isolated well before the onset of prevalent resistance serves as a therapeutic benchmark against which other strains may be classified as being susceptible or resistant to chloroquine.

This was the approach taken by Collins et al. (55) in assessing the I/CDC strain of *P. vivax* from Sumatra, Indonesia. Recrudescence of this strain occurred in all four *Aotus* and five of six *Saimiri* monkeys challenged and treated with regimens of chloroquine that were effective against the Chesson strain of *P. vivax* in the same animal system. Collins et al. (57) also characterized the XIX/CDC strain of *P. vivax* from Indonesian New Guinea in a similar system. They described a 30-mg dose (given by gavage over 3 days) as being uniformly effective against chloroquine-sensitive *P. vivax* isolates, but that standard dose against the Indonesian XIX/CDC strain failed within 28 days in 9 of 11 monkeys. These studies provided independent corroboration of resistance to chloroquine in *P. vivax*, a diagnosis that had been made using an experimental in vivo testing format.

Mechanisms and Markers

The mechanism of chloroquine activity against blood stages of *P. vivax* remains unknown. Most of what has been reported for the subject defaults to the relatively vast experimental data for *P. falciparum*, virtually all of it being derived from continuous culture systems for that parasite. The search for molecular markers of resistance in *P. vivax* thus naturally focuses upon orthologs of incriminated mutant genes and loci in *P. falciparum*, namely, *P. vivax mdr1* (Pv*mdr1*) and Pv*crt-o*. In *P. falciparum*, sequence mutations and amplification of these genes have each been linked to the resistance phenotype, and these have also been investigated using *P. vivax*.

Nomura et al. (156) found no relationship between mutations in Pv*crt-o* and either chloroquine therapeutic phenotype or accumulation of chloroquine in a novel surrogate protozoan (*Dictyostelium*, an amoeboid slime mold) in vitro model. Sa et al. (191) found no Pv*mdr1* mutations that correlated with chloroquine-resistant phenotypes. Sa et al. (192) nonetheless showed that Pv*crt-o* plays a role in chloroquine transport across membranes in an experimental system using *P. falciparum* transformed with that gene. Moreover, they applied an engineered mutant of Pv*crt-o* (threonine at position 76) in the *Dictyostelium* system and demonstrated an impaired accumulation of chloroquine relative to that of wild-type Pv*crt-o*.

Suwanarusk et al. (212) identified Y976F mutations in Pv*mdr1* as being highly prevalent among relatively large numbers of isolates (128) from a site in Indonesian Papua known to have a 65% risk of clinical resistance to chloroquine in vivax malaria compared to 69 Thai isolates, where almost uniform sensitivity to chloroquine prevails. Ninety-six percent of Indonesian isolates had this mutation, compared to 25% of Thai isolates. Those found amplified Pv*mdr1* to be more common in Thailand (21%) than Indonesia (0%) but not apparently linked to drug resistance. However, Suwanarusk et al. (213) also examined 71 and 114 *P. vivax* isolates from Thailand and Indonesia, respectively, finding increased copy numbers of Pv*mdr1* in 21% of Thai isolates and none among the Indonesian isolates. Those authors also found relatively high inhibitory concentrations of mefloquine, artesunate, and lumefantrine in ex vivo tests of Thai parasites. Taken together, their findings suggest an inverse relationship between the copy number of this gene and resistance to chloroquine and susceptibility to mefloquine, as occurs with *P. falciparum*.

Imwong et al. (110) examined Thai *P. vivax* isolates for Pv*mdr1* amplification by comparing 66 isolates from the western Thai border to 149 isolates collected elsewhere in Southeast Asia. Those authors suggested a relationship between Pv*mdr1* and the clinical use of mefloquine. Among the 16 isolates checked for the Y976F mutation in Pv*mdr1*, 1/11 and 3/5 isolates from Thailand and Myanmar, respectively, had this mutation.

When Fernandez-Becerra et al. (78) compared a patient with severe vivax malaria (responsive to quinine intravenous therapy) to another patient with uncomplicated malaria, they found amplified copy numbers of both Pv*mdr1* and Pv*crt-o* in the former patient. No studies have systematically evaluated these genes in sufficiently large series of patients with nonsevere versus severe disease.

No firm evidence yet links amplifications or polymorphisms in any gene of *P. vivax* to clinical resistance. The search to date has been limited to genes suspected in *P. falciparum* drug resistance despite early evidence suggesting distinct mechanisms at work between these species.

Distribution

Reliable surveys of resistance ascertained CQ+DCQ levels of >100 ng/ml whole blood on the day of recurrence. In the absence of recurrence, no drug levels are needed to make the diagnosis of sensitivity to chloroquine. Figure 5 represents the application of this standard to systematic surveys of chloroquine resistance in vivax malaria. The surveys, reflecting more than a decade of observations, reveal the paucity of data.

Accepting that the data provide at best a rough sketch of risk by geographic zone, it seems clear that eastern Indonesia represents the most advanced resistance problem, with a <50% probability of therapeutic success apparently being the norm (12, 14, 15, 180, 210, 211). Few data have come from nearby Papua New Guinea (87), and even less data have come from further eastward to Vanuatu. Across the western Indonesian archipelago to the Malay Peninsula, therapeutic success rates have consistently ranged between 75% and 90% (80, 81, 82, 83,

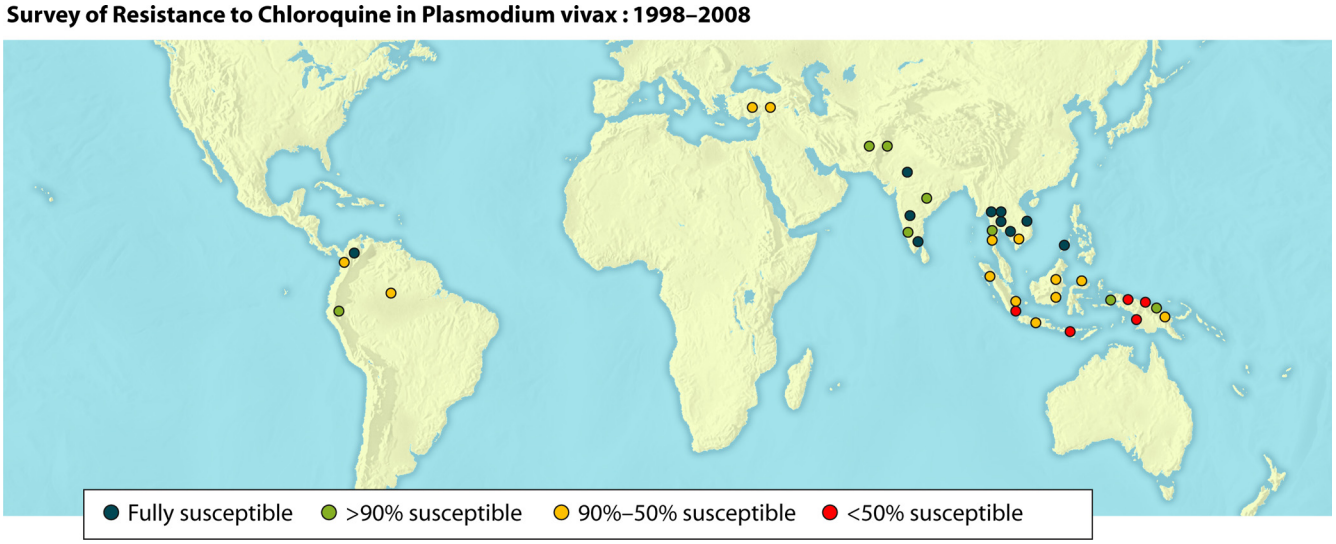

FIG. 5. Distribution of confirmed resistance to chloroquine in *P. vivax*.

220); however, a recent study in southern Sumatra found <50% treatment success (I. Sutanto, University of Indonesia, unpublished data). Recent reports from southern Myanmar and Vietnam also showed successful treatment rates within the range of 75% to 90% (95, 167, 219). A number of reliable studies involving large numbers of patients in both Thailand (59, 125, 136, 217, 229) and India have shown almost uniform susceptibility to chloroquine in vivax malaria (154, 226), although very recent work from the Gujarat State in western India found a 91% success rate among 65 evaluated patients (209). Studies in Pakistan, Afghanistan, and Azerbaijan have shown little or no evidence of resistance (121, 134, 227). Reports from Turkey showed 85% to 90% susceptibility (129), but a more recent survey from the southeastern sector showed only 78% susceptibility (130). Systematic surveys in South America showed evidence ranging from complete susceptibility to a very low risk of resistance (<5%) (38, 188), but one survey of just 27 patients found 89% susceptibility (207). A relatively recent report from Manaus, Brazil, found 10% of 109 subjects evaluated to have had apparently adequate levels of chloroquine in plasma at the time of recurrent parasitemia within 28 days (66).

In summary, the Indonesian archipelago, especially in the east, carries the highest risks of resistance of *P. vivax* to chloroquine. Most recent data also suggest an increasing risk on the nearby Malay Peninsula, including Myanmar, and eastward to southern Vietnam. With the notable exceptions of studies in southeastern Turkey, a single study in India, and one in Brazil, risk elsewhere currently appears to be low.

RESISTANCE TO PRIMAQUINE

Definition

Arnold et al. (10) induced complete resistance to the highest tolerated doses of primaquine by asexual blood stages of the *P. vivax* Marvel strain (derived from the Chesson strain). Those researchers initially administered suboptimal daily doses (22.5 mg), and through 36 sequential passages, they reached daily doses of primaquine as high as 140 mg (for adult subjects, which is well beyond normal therapeutic dosing), with no discernible impact upon asexual parasitemia. That report dealt only with blood stages of *P. vivax*, against which primaquine normally has good efficacy at therapeutic doses (174). However, primaquine is very rarely applied as a blood schizontocide against *P. vivax*, and it has very poor activity against asexual blood stages of *P. falciparum* (11). Resistance to primaquine in asexual blood stages has little therapeutic relevance, and no linkage is known for drug resistance in blood and liver stages. This review deals exclusively with the issue of resistance to primaquine in the liver stages.

Collins and Jeffery (56) pointed out the bias for both blood schizontocides and *P. falciparum* in most conventional definitions of resistance to antimalarials. A working definition of resistance to primaquine had not been put forth, much less validated. They advocated the application of the term resistance within relatively strict limits, namely, direct evidence of therapeutic failure of a given dose known to be effective within a given geographic locale or for a given strain of parasite. They pointed out that the "… characteristic requirement for higher doses of primaquine for curative effect in vivax malarias of the South Pacific and Southeast Asia is one of long standing, not one that has emerged recently." Indeed, tropical Southeast Asian strains have shown consistently poor therapeutic responses to primaquine as far back as 1944, when the Chesson strain of *P. vivax* infected an American soldier in New Guinea (Chesson was the name of the soldier) (74) and was used extensively in the subsequent development of primaquine against relapse (239).

The wartime genesis of the Chesson strain and its well-known refractoriness to primaquine merit examination in discussions of terms and their meaning in a biological sense. The term tolerant implies a permanent, long-standing, or even inherent ability to survive ordinarily effective and practical regimens of a given therapy. The fact that primaquine had not been developed in 1944 and the Chesson strain showed poor efficacy with standard primaquine therapy (15 mg daily for 14

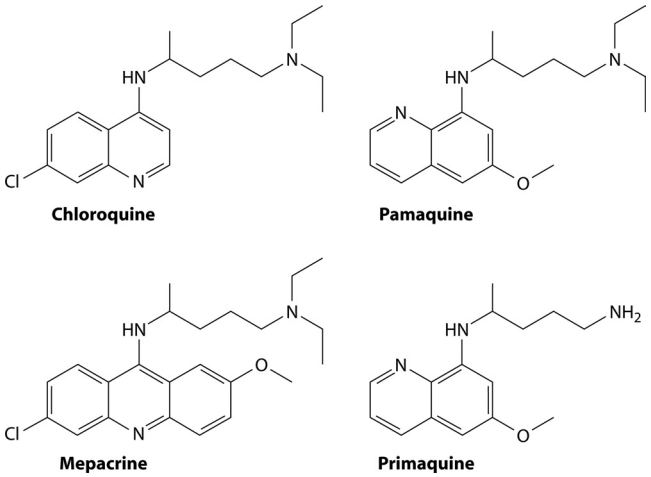

FIG. 6. Chemical structures of chloroquine, pamaquine (plasmoquin), mepacrine (atabrine), and primaquine.

days) relative to that with most temperate strains suggests an inborn tolerance of primaquine. However, the Chesson strain may have acquired resistance to 8-aminoquinolines like primaquine through selection pressures applied by therapeutic practices. From 1942 to 1945, New Guinea hosted many tens of thousands of Japanese, American, Australian, New Zealand, and Fijian troops engaged in combat for military control of the island. The strategic importance of malaria in that struggle was detailed previously (25, 68, 113).

Atebrine (also called quinocide or mepacrine) is a now obsolete 9-aminoacridine invented by the Germans in the 1930s as a synthetic replacement for quinine. The fall of Java in the Dutch East Indies to Japanese forces in early 1942 resulted in the loss of 95% of the world's quinine supply to Allied nations, and atebrine very suddenly found wide application. Its enforced use among both troops and indigenous people residing close to encampments has been documented (25, 68, 113). Atebrine was new to most doctors in 1942. According to Joy (113), "there was no published doctrine for its use, nor any information on side effects. The medical officers thus invented their own programs. These ranged from daily use of 0.1 gme to twice a week administration of 0.2 gmes, to 0.05 gmes for 5 days with 2 days of 'rest.' " The production and distribution of atebrine to forces, especially those engaged in the Southwest Pacific theater of war in 1942, had been a strategic urgency. As Joy (113) pointed out, by the standards of today, its clinical application could be characterized as experimental and reckless. There could be fewer better formulas for selecting parasites that are resistant to the drug and, perhaps, structurally related drugs as well.

The German chemists that synthesized atebrine had used the chemical structure of pamaquine as their starting point and, after examining over 12,000 compounds using bird models, found that substituting the quinoline ring with acridine provided the best therapeutic index (Fig. 6). These structurally related compounds may share some characteristics of resistance, but a highly atebrine-resistant line of *Plasmodium berghei* (M line) was cross-resistant to quinine and chloroquine but not primaquine (against blood stages) (163).

Bennett (25) shed new light on Allied antimalarial practices on New Guinea that bear more directly upon resistance to primaquine. That author described the widespread use of the original 8-aminoquinoline, pamaquine (plasmoquin) (Fig. 6), among Allied troops and, especially by American forces, among the many thousands of local inhabitants which they encountered during the progress of the war. The applied doses (20 mg daily for 5 day) were apparently intended to provide gametocytocidal activity and curb transmission. According to military historian Alan Hawk (http://ajrp.awm.gov.au/ajrp/remember.nsf/pages/NT000022CA?openDocument), Japanese troops in New Guinea also used pamaquine (plasmochin) both as prophylaxis against infection (in a single weekly dose) and as a supplement to quinine therapy. Pamaquine prevention and treatment trials were among the studies undertaken by Fairley's Australian Army Medical Research Unit at Cairns during the war (77, 214). Their focus on strategically urgent drugs lends further credence to the apparent importance and use of pamaquine for troops in the field at that time.

A circular published by the U.S. Surgeon General in 1943 (158) carried recommendations for therapeutic practice with pamaquine that acknowledged the phenomenon of the enhancement of pamaquine toxicity by atebrine. The Allies, in effect, surrendered to relapse by applying regimens of pamaquine aimed only at the elimination of gametocytes and reducing the risk of further transmission.

Atebrine and pamaquine were widely employed by military forces at war in New Guinea beginning in 1942 and persisting through 1945. The widespread application of often subtherapeutic regimens of these two drugs that were structurally related to primaquine against a pervasive malaria threat may account for the relatively poor responsiveness of the Chesson strain of *P. vivax* to antirelapse therapy with primaquine. Historical accounts of the conduct of the war in the Pacific, especially in New Guinea, support the supposition of a pervasive and persisting selection pressure favoring the emergence of *P. vivax* that is resistant to 8-aminoquinolines. Indeed, the Chesson strain proved to be far more resistant to pamaquine than to primaquine relative to the normal level of activity of each drug against other strains; whereas daily adult doses of 15 mg and 27 mg (for 14 days) of primaquine and pamaquine, respectively, prevented relapse against other strains of vivax malaria, doses of 30 mg primaquine and 90 mg pamaquine were required to achieve the same effect against the Chesson strain (5, 62). Thus, the Chesson strain of *P. vivax* may be considered to be pamaquine resistant and very likely cross-resistant to primaquine.

Collins and Jeffery (56) expressed unease with the term "resistant," apparently preferring either primaquine tolerant or primaquine refractory. They reserved use of the term for demonstrations of eroded efficacy within an area. Baseline data for sensitivity to primaquine prior to 1944 do not exist, and classification of the Chesson strain as being resistant to primaquine is thus eluded within their framework. Indeed, even today, the availability of baseline data documenting benchmark sensitivity anywhere is exceedingly rare. This constitutes the primary problem with applying the framework for defining resistance as put forth by Collins and Jeffery (56): the almost universal absence of benchmarks against which an erosion of efficacy may be measured. The task of gauging the therapeutic

utility of primaquine seems to be unmanageable, and the root of the problem lies in the interpretation of Chesson strain responsiveness to primaquine as being reflective of an inherent tolerance of the drug that may naturally vary with geography and strain.

The above-described detail afforded by the genesis of the Chesson strain provides the basis of a differing view. The army of Sergeant Chesson moved with many thousands of his fellow soldiers against many thousands of Japanese soldiers, each surrounded by thousands of indigenous Papuans. Malaria attack rates were extraordinarily high: Australian forces at Milne Bay in December 1942 suffered 4,200 malaria cases/1,000 person-years (25). Atebrine and pamaquine were the primary drugs applied against malaria. The Chesson strain of *P. vivax* should not be considered to be representative of a naturally occurring heterogeneity of sensitivity to primaquine. The evidence of circumstance instead points to a unique milieu of intense drug use and parasite exposure to those drugs, allowing the emergence of strains that were resistant to those drugs.

Defining resistance to primaquine requires setting a baseline sensitivity of *Plasmodium vivax* to the drug. The originally recommended standard therapy, a 15-mg base adult dose (or 0.25 mg/kg) once daily for 14 days, stands as an obvious candidate benchmark. This regimen provided nearly complete efficacy against strains from India, Vietnam, South Korea, and both temperate and tropical Americas (5, 49, 85, 90, 114, 148, 231). Even lower doses (10 mg/day for 14 days or 15 mg/day for 7 days) provided good efficacy (67, 221). The documented sensitivity of all strains of *P. vivax*, which was evaluated to be 15 mg daily for 14 days (adult dose), except for a single strain isolated from a soldier engaged in combat operations in New Guinea in 1944 (74), points to the use of that standard regimen as a benchmark of primaquine sensitivity for the species. This approach provides an enabling framework for gauging the encroachment of resistance to primaquine in *P. vivax.*

Diagnosis

No means for the diagnosis of resistance to primaquine by liver stages of vivax malaria has been standardized or validated. What follows represents a practical means of coping with the many nuanced ambiguities of therapeutic responses to this drug. The most important issues to address include reinfection, recrudescence (companion blood schizontocide), and compliance to prescribed therapy.

Confounding by reinfection. Evaluation of primaquine efficacy must allow for a relatively long-term follow-up of subjects for the detection of late relapse. This may be especially true in temperate zones, where late relapse is the rule, but it is also true in the tropics, where both early and late relapses occur, and these may have distinct responses to therapy (1). Moreover, more than half of subjects experienced therapeutic failure after day 28 when Alving et al. (4) evaluated 18 pamaquine analogs against relapse. This imposes a rather strict and constraining requirement for ensuring a very low probability of reinfection during the follow-up period. As discussed above, the molecular tools for classifying recurrent *P. vivax* infections as recrudescences, reinfections, or true relapses do not yet exist, and this limits meaningful evaluations to specific populations, i.e., infected travelers repatriated to nonendemic areas.

An alternative approach for coping with reinfection may be simply to measure the incidence of new infections and account for this in estimating primaquine efficacy. In a single study population randomized to three separate treatment groups, one may estimate the efficacy of any given primaquine regimen by measuring recurrences in a group given a regimen of primaquine that is certain to be effective and among those given a placebo for primaquine. This approach may account for confounding by reinfection, but statistical constraints probably limit practicality where reinfection probabilities exceed 20% per year.

Confounding by recrudescence. A potential study subject will be diagnosed with microscopically patent parasitemia with *P. vivax*. PCR diagnostics may complement that diagnosis but not supplant it, as no means of PCR diagnosis has been credibly validated with reliable estimates of specificity and sensitivity. There is no analytically valid basis for setting limits on the density of parasitemia in these subjects. Reliable evidence of parasitemia serves as simply an indicator of a likely infection of liver with hypnozoites. However, some patients may be disqualified on the basis of severe disease or an inability to adhere to oral primaquine administration.

The diagnosis comes with the obligation to treat acute disease, and a blood schizontocide must be promptly applied. The selection of that drug bears directly upon an evaluation of the efficacy of primaquine against liver stages. The use of chloroquine as a blood schizontocide is not recommended. Confirmed resistance to chloroquine by *P. vivax* leaves open the likelihood of recrudescence as a possible source of recurrent parasitemia. Nonetheless, primaquine may require chloroquine to achieve killing of hypnozoites, and the drug must be administered as a companion tissue schizontocide. The certain killing of blood stages must be accomplished with a third drug, and its selection and administration must be carefully considered with respect to yielding findings that may be reliably analyzed.

The blood schizontocide must be known to be completely efficacious against blood stages, and it must be rapidly excreted so as not to interfere with the combination of primaquine and chloroquine against liver stages. The drug must be administered upon diagnosis and almost completely eliminated before beginning the administration of primaquine and chloroquine. Good candidates for a blood schizontocide include quinine or any of the artemisinins. Each of these has evidence of good efficacy against blood stages of *P. vivax* and is rapidly excreted.

Compliance to therapy. The administration of 15 or 30 mg primaquine daily for 14 days (depending upon local practice and the objective of the survey) should commence on about day 7 after starting treatment of the blood-stage infection and should be given concurrently with a standard dose of chloroquine (10 mg/kg starting with the first dose of primaquine, followed by 10 mg/kg with the second dose and 5 mg/kg with the third dose). A subject free of risk of reinfection and given these therapies under some form of supervision is thus positioned for follow-up yielding meaningful insights with respect to the therapeutic response of liver stages of *P. vivax* to primaquine (and chloroquine).

Confounding by chloroquine in blood. The application of chloroquine and its approximately 35 days of effective levels

against *P. vivax* in blood will suppress the earliest relapses of chloroquine-sensitive *P. vivax* strains. This may bias the evaluation toward an overestimation of sensitivity to primaquine. However, the early-relapsing tropical strains of *P. vivax* also show a propensity for multiple relapses, and the emergence of primaquine-resistant hypnozoites during a year of follow-up seems highly likely. An alternative approach may be to apply quinine as both the companion tissue schizontocide and blood schizontocide. Quinine also potentiates the activity of primaquine against liver stages (6, 195).

Analysis. Follow-up duration and the interpretation of recurrent parasitemia constitute the diagnosis of resistance to primaquine. Provided that the eradication of asexual blood stages was achieved and reinfection has been reliably excluded (or measured), recurrent parasitemia may be assigned to emergence from the liver and thus represent therapeutic failure of the applied tissue schizontocide.

Almost all tropical *P. vivax* cases may be expected to relapse within a month and several times over a prolonged period (a year or more), whereas only about 20 to 40% of temperate (or Indian) *P. vivax* cases relapse at all and tend to do so only once between 6 and 12 months. The period of follow-up should be adapted to local relapse patterns. Moreover, at least one study suggested distinct therapeutic responses to primaquine (and elubaquine) by hypnozoites tending to relapse early versus late (1). The risk of relapse appeared to be higher in the short term than in the long term. Although the likelihood of confounding by recrudescence of chloroquine-resistant asexual blood forms could not be ruled out in that study, the finding bears consideration in planning an analysis of primaquine resistance. Certainly, evaluations of tropical vivax malaria should include efficacy end points at 30, 60, 120, 240, and 365 days, for example, to capture such possible differences in therapeutic responsivenesses of early compared to late hypnozoites.

The possibility exists that so-called early relapses caused by tropical Asian *P. vivax* may not be relapses but simply slow-developing primary tissue schizonts. This would very likely impact the therapeutic responsiveness to any given drug applied, i.e., targeting true hypnozoites or not (Fig. 2). However, the apparently strict >17-day pause between primary parasitemia and first recurrent parasitemia argues against that hypothesis.

Mechanisms and Markers

No mechanism or marker of resistance of liver-stage *P. vivax* to primaquine is known. Progress in this area likely awaits the successful establishment of hypnozoites in human hepatocyte cell lines in vitro, especially those allowing an analysis of the parasite transcriptome at this stage. The systematic collection of strains that are reliably phenotyped as being sensitive or resistant to primaquine in clinical studies would bring considerable analytical leverage to the ex vivo work. Genomic and proteomic studies comparing the Chesson and Korean strains of *P. vivax* may yield insights into the genetic determinants of relapse and, perhaps, susceptibility to primaquine.

Distribution

No studies have yet followed the guidelines for the diagnosis of resistance to primaquine outlined above. Objective assessments of the risk of therapeutic failure in any given region remain elusive. Nonetheless, many reports of both case and clinical studies provide a crude gauge of the geographic distribution of risk.

The broad assertion that *P. vivax* strains from Southeast Asia are less responsive to primaquine than strains from elsewhere (39, 43, 123) rests largely upon evidence from clinical trials in Thailand. Although Luxemburger et al. (137) found a 91% efficacy of 15 mg daily for 14 days with 2 months of follow-up, Walsh et al. (232) and Pukrittayakamee et al. (174) measured efficacies of that regimen to be 69% and 42%, respectively. Standard 14-day therapy of 15 mg daily failed more often than either the 22.5- or 30-mg daily regimen (34, 174), and the 22.5-mg daily regimen failed more often than did the 30-mg daily regimen (117). Likewise, a 30-mg daily dose works better when given for 11 or 14 days than when given just 5, 7, or 9 days (127). Vivax malaria acquired in Southeast Asia seems to require at least 30 mg daily for 14 days (or twice daily for 7 days) (127) to prevent relapse. In Vietnam, 1 of 28 subjects had recurrent parasitemia within 28 days after therapy with 22.5 mg primaquine twice daily for 7 days (64). Earlier studies in Vietnam (114) showed 4% relapse rates among repatriated American soldiers given supervised therapy and monitored for a year. Vivax malaria acquired in Southeast Asia very likely requires 30 mg primaquine daily for 14 days to achieve good efficacy against relapse.

The wide perception of New Guinea, the source of the Chesson strain, as harboring primaquine-tolerant or -resistant strains of *P. vivax* lacks direct substantiating data. No direct measures of the efficacy of primaquine against relapse have come from the island since the work with the Chesson strain more than 50 years ago. However, two separate large studies reported that travelers to New Guinea with vivax malaria who were treated with standard primaquine therapy were 8 to 12 times more likely to suffer relapse after primaquine therapy than patients who acquired their *P. vivax* infection anywhere else (75, 112). Vivax malaria acquired on the island of New Guinea indeed appears to be more prone to relapse after primaquine therapy than vivax malaria acquired in other regions. Effective therapy against relapse of vivax malaria acquired on the island of New Guinea may require at least 30 mg daily for 14 days, and administration of that dose for 21 days (or more) may be prudent.

Data from the Korean Peninsula, India, Pakistan, and Afghanistan also suggest that 30 mg daily for 14 days may be required for good efficacy against relapse by *P. vivax*. Park and Kang (162) measured only a 32% effectiveness of postexposure presumptive therapy with the 15-mg daily (14-day) regimen for tens of thousands of soldiers from the Republic of Korea. The 30-mg daily (14-day) regimen achieved 94% efficacy in the northwestern frontier of Pakistan (135), whereas a supervised regimen of 15 mg daily achieved only 48% efficacy in the first 3 months in the same region (133). Another study in the same region recorded a 35% efficacy for that regimen (187). Spudick et al. (208) detailed the case of an American soldier repatriated from duties in Iraq and Afghanistan with three successive

episodes of vivax malaria (two episodes requiring hospitalization and prolonged mechanical ventilation) despite administration of 30 mg daily for 14 days for each episode. In India, Rajgor et al. (178) found only 50% efficacy for the 15-mg daily regimen (14 days), but Gogtay et al. (90) found no relapses among 242 subjects thus treated in Mumbai (versus 12% relapsing without primaquine).

Risk of reinfection may confound these studies from India, Pakistan, and Afghanistan with long-term follow-up (9 to 12 months). An equal force of reinfection among placebo- and primaquine-treated groups would have the effect of diminishing estimates of efficacy in proportion to incident new infections. For example, if 20 true relapses occurred in the placebo group of 100 subjects and just 1 occurred in the primaquine treatment group of 100 subjects, the estimate of efficacy would be 95%. If during the course of 6 months of follow-up, each of the groups of 100 subjects experienced 15 new infections, the realized estimate of efficacy would be 54%. This problem can be addressed with a third treatment arm measuring the force of new infections (see above). Data from one study in Pakistan tend to support this impact in the given estimates of efficacy from that region. When Leslie et al. (135) examined the interval-specific efficacy of primaquine, the estimates were 48%, 73%, and 92% at intervals of 0 to 3 months, 4 to 6 months, and 7 to 9 months, respectively. The latter intervals would have been less likely to have reinfections due to the sharp seasonality of transmission at that study site. The available data from this region suggest a high risk of failure with the 15-mg daily regimen versus the consistently good efficacy of the 30-mg daily regimen.

Vivax malaria occurs in relative abundance in eastern Africa including Madagascar. Schwartz and Sidi (199) reported 50% attack rates among Israeli travelers to Ethiopia despite suppressive prophylaxis. A survey of 11,504 Ethiopians revealed a 1.6% prevalence of microscopically patent parasitemia caused by *P. vivax* (76). Among 4,801 cases of vivax malaria imported into Europe between 1999 and 2003, 16% of cases came from eastern and southern Africa (152). Surprisingly, 18% of those cases came from the rest of the continent despite an extremely low prevalence of detectable parasitemia in that region (63). Virtually nothing is known of the responsiveness of African *P. vivax* strains to primaquine. Smoak et al. (204) described 60 American soldiers developing vivax malaria after repatriation from Somalia and being treated with unsupervised standard primaquine therapy: 26 soldiers (43%) relapsed.

The available data on primaquine efficacy from South America point to a similarly poor performance of the 15-mg standard regimen. Duarte et al. (70) managed 50 patients with vivax malaria through a supervised 15-mg daily regimen (14 days) and 6 months of follow-up in an area without risk of reinfection: 7 of those patients experienced relapse. It was not possible to calculate efficacy without a placebo control, but the 14% relapse rate among patients given supervised therapy strongly suggests an inadequacy of the regimen. Alvarez et al. (3) monitored 210 subjects with vivax malaria for 6 months after therapy with total primaquine doses of 45, 105, and 210 mg in a region of Colombia where malaria is endemic. Relapse occurred in 45%, 37%, and 18% of these subjects, respectively. Setting aside the issue of confounding by reinfection (as discussed above) and applying the 45-mg treatment group as being representative of a placebo control group yielded an estimate of efficacy of 60%. Villalobos-Salcedo et al. (230) compared a standard 14-day regimen of 15 mg primaquine with a 5-day regimen among 61 adults with vivax malaria in Brazil monitored for 60 days after treatment. Again setting aside confounding by reinfection and supplanting a placebo control with the 5-day treatment group yielded an estimate of efficacy of 76%. Arias and Corredor (9) detailed 11 cases of relapse following standard primaquine therapy in Colombia. Gascon et al. (86) described two cases of primaquine failure from Guatemala who were treated in Spain. Finally, Nayar et al. (155) described the primaquine-resistant Brazil I/CDC strain of *P. vivax* coming from a traveler infected in Brazil who failed three separate courses of 15 mg daily for 14 days but finally succeeded with that regimen after it was administered over 28 days. Vivax malaria from South America appears to respond poorly to the 15-mg (14-day) regimen, and the 30-mg regimen should be applied.

RESISTANCE TO ANTIFOLATES

Malariologists have long known *P. vivax* to be intrinsically capable of developing resistance to pyrimethamine relatively quickly (104). The most widely applied antimalarial in this class of drugs was the fixed combination of SP, targeting parasite dihydropteroate synthase and dihydrofolate reductase (DHFR), respectively. Hawkins and colleagues (101) constructed a history suggesting that the early perception of poor efficacy against asexual blood stages may have been a product of confusion with the failure of either drug to prevent relapse in *P. vivax*. Those authors pointed to an exceptional study of the effect of pyrimethamine on the Chesson strain of *P. vivax* conducted by Coatney et al. (48) that characterized the activity of pyrimethamine against this parasite as being superior. Hawkins et al. (101) contrasted that with data from a report by Young and Burgess (241) emphasizing the intrinsic susceptibility to induced resistance to pyrimethamine. Those investigators also linked the clinical failure of SP against *P. vivax* to the accumulation of multiple mutations in DHFR, with wild-type DHFR isolates showing in vivo sensitivity to SP therapy (97, 109), along with in vitro evidence of a similar process in transfected *Saccharomyces cerevisiae* systems (97, 98).

The picture that emerges tends to undermine the perception of *P. vivax* as being intrinsically not responsive to SP therapy. The broad deployment of SP against *P. falciparum* would have exerted incidental selection pressure for DHFR/dihydropteroate synthase mutations in *P. vivax*. Available evidence points to the acquisition of a sufficient number of mutations in DHFR to create a high probability of therapeutic failure against *P. vivax*. The likely persistence of some wild-type DHFRs among *P. vivax* isolates, however, does not necessarily argue for the deliberate application of SP against this infection. The broad lack of technological capacity in areas of endemicity would almost always preclude genetic screening prior to SP therapy. The importance of the distinctions pointed out by Hawkins et al. (101) instead lies in the realization that the machinery of folate metabolism in *P. vivax* represents potentially highly vulnerable targets of chemotherapy and merits exploration in drug development.

GAMETOCYTOCIDAL THERAPIES

The above-described presentation of chloroquine as being an ideal antimalarial drug comes with an important caveat, which is its failure to adversely affect the gametocytes of *P. falciparum*, and it apparently even enhances the likelihood of transmission, especially for chloroquine-resistant strains (35, 107). This biology may have had a direct role in the nearly complete proliferation of chloroquine-resistant falciparum malaria that occurred between 1945 and 1985.

The same biology is not true for *P. vivax* and may likewise at least partially explain the relatively slow appearance and spread of chloroquine-resistant *P. vivax*. Jeffery (111) provided conclusive evidence that either standard oral or intramuscular chloroquine therapy against the Chesson strain of *P. vivax* caused an abrupt and complete halt to infectivity to *Anopheles quadrimaculatus* mosquitoes among 20 gametocytemic human subjects. Nonetheless, *P. vivax*, like most other plasmodia and unlike *P. falciparum*, undergoes gametocytogenesis relatively early during blood-stage development. *P. vivax* gametocytes may appear in the bloodstream before the onset of symptoms of acute malaria (30, 96) and perhaps provide the opportunity for transmission before treatment of any kind is administered. However, in a series of 152 neurosyphilis patients challenged with the St. Elizabeth (North American) strain of *P. vivax*, McKenzie et al. (144) found that fever preceded gametocytemia by several days.

If the transmission of vivax malaria occurs in asymptomatic (and untreated) hosts, the parasite will not directly experience drug selection pressure, and drug sensitivity would likely prevail. However, as demonstrated by the *P. falciparum*-chloroquine example, the application of a therapy that does not eliminate infectious gametocytes may have long-term consequences with severe public health impacts. Peters et al. (166) used a rodent malaria system to demonstrate the ability of a gametocytocide to protect the blood schizontocide from the onset of resistance. The evaluation of new therapies for *P. vivax* should consider the important issue of gametocytocidal properties.

Butcher (35) reviewed various approaches to assessing gametocytocidal activity and the available data up to 1997. That author explained the technical basis for considering human subjects infected and infectious to directly fed mosquitoes as the ideal system for ascertaining gametocytocidal activity. Studies by Jeffery (111) represent this ideal system. Klein and colleagues (118) approached this ideal using *P. vivax*-infected subjects given standard therapies but used membrane feeding of mosquitoes. They showed chloroquine and primaquine combined required at least 5 h to affect transmissibility. Ex vivo and/or in vitro systems present pitfalls in interpretation but may be ideally suited to the screening of relatively large numbers of experimental drugs in preclinical evaluations, as Coleman et al. (53) demonstrated with seven compounds against *P. vivax.* The same group (169) applied a similar system to demonstrate the transmission-blocking activity of tafenoquine and artelinic acid (as metabolized in mice) against *P. vivax* in *Anopheles dirus* mosquitoes.

Studies of gametocyte burdens following therapy may be the least informative approach but certainly represent the most accessible means of analysis. As Butcher (35) pointed out, the presence of gametocytes and their densities may poorly correlate with the variable of interest, i.e., infectiousness to mosquitoes. Nonetheless, a demonstration of the time required for the elimination of gametocytemia may provide a basis for useful comparisons among drugs. Pukrittayakamee et al. (177) recently compared times to elimination of patent *P. vivax* gametocytemia among 349 patients treated with 16 different therapies. The demonstrated very rapid elimination of gametocytes by both artesunate and artemether (1 to 2 days) provides reassurance of the ability of this class of drugs to reduce the risk of transmission after therapy. Nacher and colleagues (153) reached essentially similar conclusions by analyzing 1,487 patients with vivax malaria treated with either chloroquine or artesunate.

RESISTANCE AND CONTROL OF VIVAX MALARIA

The neglect of vivax malaria over the past decades positions this parasite for a high probability of persistence in the face of attempts to attack it with currently available tools. The outlook for successful intervention against dormant hypnozoites looks especially bleak. This weakness may be as operationally crippling to control efforts as having no access to insecticides, bed nets, diagnostics, or chemoprophylactics. Primaquine represents our only currently available weapon against the hypnozoite reservoir. That drug, in service for almost 60 years, may be failing and may not work as prescribed; recommended regimens require 2 weeks of daily dosing; and the drug may cause potentially fatal hemolytic anemia in some people with a G6PD deficiency. We do not know how the drug works, if it works, how it fails, or how it threatens G6PD-deficient persons. The failure to improve or replace primaquine secures the position of vivax malaria as a persistent and severe challenge to public health. The recent expression of the sentiment that we possess the tools to eradicate malaria has been made without consideration of the problem of vivax malaria.

Control or elimination may require the deployment of fixed-dose combined therapies that are effective against drug-resistant *P. falciparum* and *P. vivax* isolates, including the liver stages of the latter and very likely the sexual blood stages of both. The effectiveness of therapeutic practice in areas of endemicity will not bear up under the weight of complex and sometimes toxic regimens for each species and stage of plasmodia. The challenge to field such a medicine includes safety among people lacking access to screening against contraindications. The absence of such a tool argues against the expressed adequacy of available tools for control, elimination, and certainly eradication.

Mass drug administration (MDA) is the practice of distributing therapy for malaria to all people in a given area regardless of symptoms or any diagnostics. The appropriate application of MDA combined with other control strategies has been a highly effective tool. The practice fell into disfavor for decades, largely thanks to what proved to be inappropriate MDA strategies like the distribution of table salts containing antimalarial drugs. Greenwood (91) recently described the attributes of an appropriate application of MDA, and the discussion is highly relevant to the control and eradication of vivax malaria. In the absence of a transmission-blocking vaccine, MDA may be the method of choice for ensuring that asymptomatic indi-

viduals in a given population do not infect mosquitoes before seeking treatment.

The ideal drug or set of drugs for MDA will be delivered as a single dose with assurance of reasonable safety in the absence of clinical or laboratory screening for contraindicating risk factors. The drug(s) will have efficacy against sexual and asexual forms (including liver) and will persist in the bloodstream at protective levels for at least 2 months. The latter characteristic permits the killing of parasites that are resident in anopheline hosts at the time of MDA, i.e., delivering the parasites into a host population that remains inhospitable to parasite development for a period that exceeds the life span for most anopheline vectors. Such a drug(s) does not yet exist. That may be a consequence of severely constricting technological barriers or the failure of the community of drug developers (private and public) to conceptually reach beyond singular therapeutic goals for specific drugs or combinations of drugs. It is at least safe to say that few institutions have come to the development table with the goal of fielding a safe and effective agent of MDA for the elimination of malaria from a population.

One drug, perhaps unwittingly, has approached this ideal. Tafenoquine has shown activity against all stages of plasmodia at therapeutic doses and has a very long plasma half-life that exerts effective chemoprophylaxis for 2 to 3 months (201). It remains at least conceptually possible that tafenoquine may provide the ideal tool for MDA and eradication operations (165). However, at least preliminary clinical evidence suggests that tafenoquine may not be safe for people with G6PD deficiency. That would rule out significant proportions of many endemic populations including all women who happen to be pregnant at the time of MDA. 8-Aminoquinoline toxicity to G6PD-deficient persons may thus be the single greatest obstacle to the fielding of the ideal agent of MDA.

More than 50 years after the linked discoveries of both primaquine and G6PD deficiency, an understanding of the mechanism of that toxicity and how to cope with it in drug development remains elusive (32). The neglect of this specific and relatively simple biochemical problem, as one part of the far-broader neglect of *P. vivax*, may prove more costly than all others. It may be the primary obstacle to advancing from where we stand today, with a mixed bag of variably safe drugs that are effective against some stages or species but not others, to fielding the ideal antimalarial therapy for MDA. Fielding such a therapy would almost certainly deliver a measurable blow against plasmodia, and it would lend credence and confidence to a serious discussion of global eradication. However, we are currently burdened under drug testing, evaluation, and licensing paradigms designed and equipped to cope with falciparum malaria. The far more nuanced problem of *P. vivax* may require renovation of that infrastructure.

FUTURE PERSPECTIVES

Vivax malaria more seriously threatens more people than has been historically appreciated. The inadequacy of the available analytical and therapeutic tools for coping with this threat constitutes the fallout of a long neglect. Work must focus on addressing these gaps in order to field tools that are capable of impacting the huge burden on public health imposed by *P. vivax*. The following research priorities represent the means to a very wide range of urgently needed research products in clinical medicine, biology, genomics, immunology, pharmacology, and epidemiology: development of geostatistically robust means of virtual real-time mapping of the risk and burden of vivax malaria; discovery of a practical means for the continuous cultivation of *P. vivax* in vitro; discovery of a practical means for generating viable hypnozoites in tissue culture systems; validation of practical in vitro techniques for ascertainment of sensitivity of *P. vivax* blood and liver stages to drugs; validation of practical in vivo techniques for ascertainment of sensitivity of *P. vivax* blood and liver stages to drugs; establishment of preclinical research centers having access to infected patients, suitable nonhuman primates, and insectaries for anopheline mosquitoes; establishment of clinical research centers in nonendemic areas which are capable of routinely and safely conducting human challenge trials with *P. vivax* sporozoites and blood stages; and establishment of clinical research centers having access to patients with vivax malaria who are not exposed to reinfection during long periods of follow-up.

The creation of these essential tools may be required for the discovery, clinical evaluation, and licensing of a single, fixed-dose combination of drugs that is capable of the safe and well-tolerated elimination of all stages of falciparum and vivax malarias when administered over a brief period without the necessity of clinical supervision. The same assets would also prove essential to parallel and vitally important efforts to field practical vaccines.

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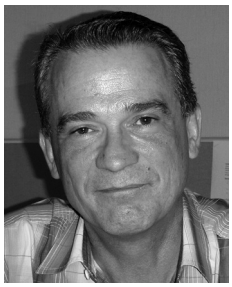

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